ROBUST SUMMARY
STYRENE, AR-METHYL-(VINYL TOLUENE)
CAS # 25013-15-4

HUMAN HEALTH EFFECTS ELEMENTS
Acute Toxicity – Study 1

Test Substance

Remarks: Vinyl Toluene (Mixed Isomers)
Purity: Approximately 98% (combined 55%-70% meta-isomer and 30%-45% para-isomer) obtained by ultraviolet or infrared analysis
Solvent carrier: Olive oil or corn oil solution emulsified with a 5-10% aqueous solution of acacia (gum arabic)
Contaminants: None reported
Chemical formula: C_9H_{10}

Method

Method/Guideline: Not referenced
GLP: Unknown
Year of study: 1956
Species: Rat
Strain: Wistar
Sex: Male
Number per dose: Not reported
Vehicle: Olive oil or corn oil solution emulsified with a 5-10% aqueous solution of acacia (gum arabic). The total volume administered was never greater than 7 cc.
Route of Administration: Single oral dose
Remarks: Male Wistar rats (175 to 250 grams; 42 animals used) were used in a single oral dose study. The test substance was introduced into the stomach by means of a stomach tube (8FS 16 inch all-rubber catheter), either as the undiluted material or as an olive-oil or corn-oil solution emulsified with a 5-10% aqueous solution of acacia (gum arabic). The total volume administered was never greater than 7 cc. All the surviving rats were observed until recovery was assured (usually about two weeks).

Results

Acute Lethal Value: 4.0 g/kg
Remark: When the rats were autopsied, slight liver changes and, in some instances, some kidney involvement of questionable significance was observed.

Conclusions: Vinyl toluene exhibits low acute oral toxicity. The approximate LD_{50} in male rats is 4.0 g/kg.

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HUMAN HEALTH EFFECTS ELEMENTS
Acute Toxicity – Study 1

Data Quality: Valid with restrictions

Remarks: Several experimental details (i.e., doses used, number of animals per dose, statistical methodology, detailed sublethal observations, etc.) were not reported in the study.

Reference:

Wolf, M.A., Rowe, V.K., McCollister, D.D., Hollingsworth, R.L., and Oyen, F. (1956) Toxicological studies of certain alkylated benzenes and benzene. Am. Med. Assoc. Arch. Ind. Health 14:387-398.

ROBUST SUMMARY
STYRENE, AR-METHYL-(VINYL TOLUENE)
CAS # 25013-15-4

HUMAN HEALTH EFFECTS ELEMENTS
Acute Toxicity – Study 2

Test Substance

Remarks:	Vinyl Toluene (Mixed Isomers) Purity: Approximately 98% (combined 55%-70% meta-isomer and 30%-45% para-isomer) obtained by ultraviolet or infrared analysis Solvent carrier: None Contaminants: None reported Chemical formula: C_9H_{10}

Method

Method/Guideline:	Adams *et al*., 1941
GLP:	Unknown
Year of study:	1956
Species:	White rabbits, heterogeneous stock raised in the laboratory
Strain:	Not reported
Sex:	Not reported
Number per dose:	Not reported
Vehicle:	None
Route of Administration:	Dermal
Remarks:	Routinely, 10 to 20 applications of the undiluted vinyl toluene were made to the ear and a like number bandaged onto the shaved abdomen over a period of two to four weeks. The animals were observed daily and were weighed weekly. A significant loss of weight or signs of abnormal behavior were interpreted as indicative of absorption through the skin in acutely toxic amounts.

Results The degree of irritation was slight to moderate defined as perceptible to definite erythema. The degree of necrosis was moderate defined as the development of edema and superficial necrosis. This resulted in a "chapped" appearance and exfoliation of large patches of skin. As judged by gross appearance, behavior, and body weight of rabbits during the skin-irritation tests, there was no indication that the test substance was absorbed through the skin in acutely toxic amounts.

Conclusions: Vinyl toluene exhibited moderate skin irritation and blistering in repeated dermal exposure to white rabbits.

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CAS # 25013-15-4

HUMAN HEALTH EFFECTS ELEMENTS
Acute Toxicity – Study 2

Data Quality: Valid with restrictions

Remarks: Several experimental details (i.e., number of animals used per dose, statistical methodology, sublethal observations, etc.) were not reported in the study.

Reference:

Wolf, M.A., Rowe, V.K., McCollister, D.D., Hollingsworth, R.L., and Oyen, F. (1956) Toxicological studies of certain alkylated benzenes and benzene. Am. Med. Assoc. Arch. Ind. Health 14:387-398.

Adams, E.M., Irish, D.D., Spencer, H.C., and Rowe, V.K. 1941 Response of rabbit skin to compounds reported to have caused acneform dermatitis. Indust. Med. 2:1-4.

ROBUST SUMMARY
STYRENE, AR-METHYL-(VINYL TOLUENE)
CAS # 25013-15-4

HUMAN HEALTH EFFECTS ELEMENTS
Acute Toxicity – Study 3

Test Substance

Remarks:	Vinyl Toluene (Mixed Isomers) Purity: Approximately 98% (combined 55%-70% meta-isomer and 30%-45% para-isomer) obtained by ultraviolet or infrared analysis Solvent carrier: None Contaminants: None reported Chemical formula: C_9H_{10}

Method

Method/Guideline:	Not reported
GLP:	Unknown
Year of study:	1956
Species:	White rabbits, heterogeneous stock raised in the laboratory
Strain:	Not reported
Sex:	Not reported
Number per dose:	Not reported
Vehicle:	None
Route of Administration:	Eye
Remarks:	The effect of the test substance tested upon the rabbit's eye was ascertained by introducing two drops of liquid onto the right eyeball. Visual observations of irritation and corneal injury (both internal and external) were made upon the treated eye at the following times after treatment: three minutes; one hour; and one, two, and seven days. A 5% water solution of fluorescein dye was used to stain and render visible the external injury of the cornea after the first three minutes.

Results Vinyl toluene caused slight conjunctival irritation but did not damage the cornea. The dose was estimated at 90 mg.

Conclusions: Vinyl toluene caused mild irritation to the rabbit eye.

Data Quality: Valid with restrictions

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CAS # 25013-15-4

HUMAN HEALTH EFFECTS ELEMENTS
Acute Toxicity – Study 3

Remarks: Several experimental details (i.e., number of animals per dose, statistical methodology, sublethal observations, etc.) were not reported in the study.

Reference: Wolf, M.A., Rowe, V.K., McCollister, D.D., Hollingsworth, R.L., and Oyen, F. (1956) Toxicological studies of certain alkylated benzenes and benzene. Am. Med. Assoc. Arch. Ind. Health 14:387-398.

ROBUST SUMMARY
STYRENE, AR-METHYL-(VINYL TOLUENE)
CAS # 25013-15-4

HUMAN HEALTH EFFECTS ELEMENTS
Repeated Dose Toxicity – Study 1

Test Substance

Remarks:	Vinyl Toluene (Mixed Isomers) Purity: Approximately 99% (combined 65%-71% meta-isomer and 32%-35% para-isomer) by gas chromatography Solvent carrier: None Contaminants: None of the potential degradation products was determined to be present at concentrations greater than 0.05% of the VT concentration. Chemical formula: C_9H_{10}

Method

Method/Guideline:	Study was performed under the direction of the NIEHS and conducted in compliance with National Toxicology Program (NTP) protocols. EPA OPPTS Method 870.3465 is a comparable method.
GLP:	Yes; reviewed and approved by the NTP Board of Scientific Counselor's Peer Review Panel.
Year of study:	1990
Species:	Rat
Strain:	F344/N
Sex:	Both
Number:	5 males and 5 females
Route of Exposure:	Inhalation
Exposure period:	15 days
Frequency:	6h/day for 10 d over 15 d
Doses:	0, 200, 400, 800, and 1,300 ppm
Control:	Yes
Post observation:	All animals sacrificed after 15 d exposure.
Statistical Method:	ANOVA with Dunnett's test for multiple comparisons of organ and body weights.
Remarks:	Groups of five F344/N rats (7 to 8 weeks) (Charles River Breeding Laboratories) of each sex were exposed to air concentrations of vinyl toluene (mixed isomers) at 0, 200, 400, 800, and 1,300 ppm for 6 hours per day for 10 days over a 15-day period. Male body weights ranged from 142 to 148 grams and females ranged from 110 to 114 grams. VT was generated by using either a J-tube or a gas dispersion-type system in which heated air was passed through liquid VT. The concentration of VT in the chambers and the exposure room was monitored by an automatic sampling system

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coupled to a gas chromatograph (Varian 2700) equipped with a flame ionization detector and a 3% SP2250 column. Rats were observed once per day and were weighed before exposure, after 1 week, and at the end of the studies. A necropsy was performed on all animals. Histopathologic examinations were performed on all animals in the high dose group. Tissue examined include:brain, duodenum, heart, kidneys, lungs, pancreas, and stomach. Brain and liver were weighed at necropsy.

Results

NOAEL dose:	Male rats, 200 ppm; female rats <200 ppm
NOAEL effect:	Decrease in necropsy weight (males and females) and an increase in liver weight/body weight (females only) relative to controls.
LOAEL dose:	Male rats, 400 ppm; female rats ≤ 200 pm
LOAEL effect:	Decrease in necropsy weight (males and females) and an increase in liver weight/body weight (females only) relative to controls.
Statistical results:	Significant at $p<0.01$ (body weight) and $p<0.05$ (liver weight/body weight, females only).
Results Remarks:	All rats lived to the end of the study. Lethargy, excessive lacrimation, and red-staining (porphyrin) material around the nose and the mouth were observed for rats exposed to 1,300 ppm. The mean body weights at necropsy of male rats exposed to 400-1,300 ppm were 13-19% significantly lower than that of the controls and female rats exposed to 200-1,300 ppm were 8-13% significantly lower than that of controls. Absolute and relative liver weights were significantly increased for rats exposed to 1,300 ppm. Four of five male rats exposed to 1,300 ppm had centrilobular necrosis and focal inflammatory cell infiltration of the liver. Minimal to slight centrilobular vacuolization of the liver was seen in all female rats exposed to 1,300 ppm. Dysplasia of the bronchial epithelium, chronic bronchitis, and lymphoid hyperplasia of the lung were observed in all rats exposed to 1,300 ppm. The severity was minimal to slight in males and minimal in females. Mean (± SE) male body weights (grams; *indicates statistically significant from controls) for 0, 200, 400, 800, and 1,300 ppm were 211 (3), 203 (2), 183 (3)*, 183 (3)*, and 171 (2)*, respectively. Male liver weights (grams) were 11.5 (0.40), 11.5 (0.180), 9.8 (0.23)*, 11.5 (0.27), and 12.5 (0.18)*, respectively. Male liver weight/body weight ratios (mg/g) were 54 (1.8), 57 (0.4), 53 (0.4), 63 (0.4)*,

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and 73 (0.9)*, respectively. Mean (± SE) female body weights (grams; *indicates statistically significant from controls) for 0, 200, 400, 800, and 1,300 ppm were 144 (1), 132 (1)*, 129 (1)*, 131 (1)*, and 125 (1)*, respectively. Female liver weights (grams) were 7.4 (0.13), 7.1 (0.13), 5.7 (0.09)*, 6.8 (0.13)*, and 8.6 (0.18)*, respectively. Female liver weight/body weight ratios (mg/g) were 51 (0.4), 54 (0.9)*, 44 (0.4)*, 52 (0.4), and 69 (1.3)*, respectively.

Conclusions

Because of decreased weight gain at 1,300 ppm (19% for males, 13% for females), the high concentration selected for the 13-week studies was 1,000 ppm.

Data Quality

Reliability: Acceptable

Remarks: All key parameters (i.e., exposure conditions, number of animals, observations, etc.) were appropriate and adequately described in the study.

Reference

Toxicology and Carcinogenesis Studies of Vinyl Toluene (Mixed Isomers; 65-71% meta-isomer and 32-35% para-isomer) in F344/N Rats and B6C3F$_1$ Mice (Inhalation Studies). National Toxicology Program Technical Report Series No. 375, NIH Publication No. 90-2830.

ROBUST SUMMARY
STYRENE, AR-METHYL-(VINYL TOLUENE)
CAS # 25013-15-4

HUMAN HEALTH EFFECTS ELEMENTS
Repeated Dose Toxicity – Study 2

Test Substance

Remarks:	Vinyl Toluene (Mixed Isomers) Purity: Approximately 99% (combined 65%-71% meta-isomer and 32%-35% para-isomer) by gas chromatography Solvent carrier: None Contaminants: None of the potential degradation products was determined to be present at concentrations greater than 0.05% of the VT concentration. Chemical formula: C_9H_{10}

Method

Method/Guideline:	Study was performed under the direction of the NIEHS and conducted in compliance with National Toxicology Program (NTP) protocols. EPA OPPTS Method 870.3465 is a comparable method.
GLP:	Yes; reviewed and approved by the NTP Board of Scientific Counselor's Peer Review Panel.
Year of study:	1990
Species:	Mouse
Strain:	$B6C3F_1$
Sex:	Both
Number:	5 males and 5 females
Route of Exposure:	Inhalation
Exposure period:	15 days
Frequency:	6h/day for 10 d over 15 d
Doses:	0, 10, 25, 50, 100, and 200 ppm
Control:	Yes
Post observation:	All animals sacrificed after 15 d exposure.
Statistical Method:	ANOVA with Dunnett's test for multiple comparisons of organ and body weights.
Remarks:	Groups of five $B6C3F_1$ mice (9 to 10 weeks old) (Charles River Breeding Laboratories) of each sex were exposed to air concentrations of vinyl toluene (mixed isomers) at 0, 10, 25, 50, 100, and 200 ppm for 6 hours per day for 10 days over a 15-day period. Male body weights ranged from 24 to 27 grams and females ranged from 21.2 to 23.4 grams. VT was generated by using either a J-tube or a gas dispersion-type system in which heated air was passed through liquid VT. The concentration of VT

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in the chambers and the exposure room was monitored by an automatic sampling system coupled to a gas chromatograph (Varian 2700) equipped with a flame ionization detector and a 3% SP2250 column. Mice were observed once per day and were weighed before exposure, after 1 week, and at the end of the studies. A necropsy was performed on all animals. Histopathologic examinations were performed on all animals in the high dose group and on 1 male and 1 female in mouse control groups. Tissue examined include:brain, duodenum, heart, kidneys, lungs, pancreas, and stomach. Brain and liver were weighed at necropsy.

Results

NOAEL dose:	100 ppm
NOAEL effect:	Decrease in male survival and increase in liver weight (males) and liver weight/body weight (males and females) relative to controls.
LOAEL dose:	200 ppm
LOAEL effect:	Decrease in survival and increase in liver weight (males) and liver weight/body weight (males and females) relative to controls.
Statistical results:	Significant at $p<0.01$.
Results Remarks:	Three of five male mice exposed to 200 ppm died before the end of the study. Compound-related clinical signs observed at 200 ppm included lethargy and palpebral closure. Ataxia was seen at 100 ppm. There was no correlation between the exposure concentration and change in mean body weights. The absolute and relative liver weights were increased for mice exposed to 200 ppm. One control mouse and five mice of each sex exposed to 200 ppm were examined microscopically. Severe hyperemia and hemorrhage of the pulmonary parenchyma were seen in exposed male mice that died on day 3. Three other exposed male mice had interstitial pneumonia. Four of five male mice exposed to 200 ppm had moderate to severe necrosis of the liver; all five female mice exposed to 200 ppm had hyperplasia of the epithelium of the intrapulmonary bronchi and centrilobular hepatocellular necrosis, vacuolization, and polymorphonuclear leukocyte infiltrates in the liver. Mean (± SE) male body weights (grams; *indicates statistically significant from controls) for 0, 10, 25, 50, 100, and 200 ppm were 26.4 (0.45), 27.8 (0.09)*, 27.6 (0.18)*, 26.0 (0.31),

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27.2 (0.31), and 26.5 (0.35), respectively. Male liver weights (grams) were 1.36 (0.036), 1.5 (0.040), 1.53 (0.031)*, 1.47 (0.031), 1.51 (0.063), and 1.72 (0.0)*, respectively. Male liver weight/body weight ratios (mg/g) were 52 (1.34), 54 (1.34), 56 (1.34), 57 (0.89), 55 (1.79), and 65 (0.71)*, respectively. Mean (± SE) female body weights (grams; *indicates statistically significant from controls) for 0, 10, 25, 50, 100, and 200 ppm were 24.8 (0.09), 23.6 (0.45), 24.8 (0.40), 22.6 (0.31)*, 23.8 (0.27), and 23.6 (0.45), respectively. Female liver weights (grams) were 1.41 (0.018), 1.22 (0.040)*, 1.22 (0.013)*, 1.36 (0.022)*, 1.20 (0.022)*, and 1.52 (0.054), respectively. Female liver weight/body weight ratios (mg/g) were 57 (0.89), 52 (1.34)*, 49 (0.45)*, 60 (0.45), 50 (0.89)*, and 64 (1.34)*, respectively.

Conclusions

Three of five male mice exposed to 200 ppm VT died before the end of the study. Four of five male mice exposed to 200 ppm had moderate to severe hepatocellular necrosis; all female mice exposed to 200 ppm had hyperplasia of the epithelium of the intrapulmonary bronchi and centrilobular necrosis, vacuolization, and inflammatory cell infiltrates in the liver.

Data Quality

Reliability: Acceptable

Remarks: All key parameters (i.e., exposure conditions, number of animals, observations, etc.) were appropriate and adequately described in the study.

Reference

Toxicology and Carcinogenesis Studies of Vinyl Toluene (Mixed Isomers; 65-71% meta-isomer and 32-35% para-isomer) in F344/N Rats and B6C3F$_1$ Mice (Inhalation Studies). National Toxicology Program Technical Report Series No. 375, NIH Publication No. 90-2830.

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STYRENE, AR-METHYL-(VINYL TOLUENE)
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HUMAN HEALTH EFFECTS ELEMENTS
Repeated Dose Toxicity – Study 3

Test Substance

Remarks:	Vinyl Toluene (Mixed Isomers) Purity: Approximately 99% (combined 65%-71% meta-isomer and 32%-35% para-isomer) by gas chromatography Solvent carrier: None Contaminants: None of the potential degradation products was determined to be present at concentrations greater than 0.05% of the VT concentration. Chemical formula: C_9H_{10}

Method

Method/Guideline:	Study was performed under the direction of the NIEHS and conducted in compliance with National Toxicology Program (NTP) protocols. EPA OPPTS Method 870.3465 is a comparable method.
GLP:	Yes; reviewed and approved by the NTP Board of Scientific Counselor's Peer Review Panel.
Year of study:	1990
Species:	Rat
Strain:	F344/N rats
Sex:	Both
Number:	10 males and 10 females
Route of Exposure:	Inhalation
Exposure period:	13 weeks
Frequency:	6h/day for 64 d over 13 weeks
Doses:	0, 25, 60, 160, 400, and 1,000 ppm
Control:	Yes
Post observation:	All surviving animals were sacrificed after 13 week exposure.
Statistical Method:	ANOVA with Dunnett's test for multiple comparisons for organ weights.
Remarks:	Thirteen-week studies were conducted to evaluate the cumulative toxic effects of repeated inhalation of vinyl toluene and to determine the concentrations to be used in the 2-year studies. Male and female F344/N rats (7 to 8 weeks old) were observed for 20 days and assigned to groups according to tables of random numbers. Feed was available *ad libitum* during nonexposure periods; water was available at all times. Groups of 10 rats of each sex were exposed to air concentrations of VT at target

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HUMAN HEALTH EFFECTS ELEMENTS
Repeated Dose Toxicity – Study 3

concentrations of 0, 25, 60, 160, 400, or 1,000 ppm, 6 hours per day, 5 days per week for 13 weeks (64 exposures). VT vapor was generated by using either a J-tube or a gas dispersion-type system in which heated air was passed through liquid VT. The vapor then entered the airstream near the top of the chamber and was mixed in the chamber plenum before entering the exposure area of the chamber. The concentration of VT in the chambers and the exposure room was monitored by an automatic sampling system coupled to a gas chromatograph (Varian 2700) equipped with a flame ionization detector and a 3% SP2250 column. Uniformity of vapor concentration in each exposure chamber and at each position with animals present was measured periodically throughout the studies by the same system used for daily concentration monitoring to validate the use of single-port sampling for daily concentration monitoring. The coefficients of variation were always found to be less than 10%.

Animals were observed one or two times per day; moribund animals were killed. Clinical signs were recorded once per week. Individual animal weights were recorded once per week. At the end of the 13-week study, survivors were killed. A necropsy was performed on all animals except those excessively autolyzed or cannibalized. Histologic examinations were performed on all rats in the control and 1,000 ppm groups. Tissues examined include: adrenal glands, bone, brain, cecum, colon, duodenum, epididymis/seminal vesicles/prostate/testes or oviduct/ovaries/uterus, esophagus, heart, ileum, jejunum, kidneys, larynx, liver, lungs, mammary gland, mandibular and mesenteric lymph nodes, mesentery, nasal passage, pancreas, parathyroid glands, pituitary gland, preputial or clitoral gland, rectum, salivary glands, skin, spinal cord, spleen, stomach, thymus, thyroid gland, trachea, and urinary bladder.

Results

NOAEL dose:	60 ppm
NOAEL effect:	Mild nephropathy characterized by increased tubular casts in male rats.
LOAEL dose:	160 ppm

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LOAEL effect: Mild nephropathy characterized by increased tubular casts in male rats.

Statistical results: Significant at $p<0.01$ for body weights and liver weight/body weight.

Results Remarks: All rats lived to the end of the study. The final mean body weights of male rats exposed to 160, 400, and 1,000 ppm were 6, 8, and 19% lower, respectively, compared to controls. The final mean body weights of female rats exposed to 160, 400, and 1,000 ppm were 5, 6, and 12% lower, respectively, compared to controls. Excessive lacrimation, palpebral closure, and rough hair coats were observed in rats exposed to 1,000 ppm. Relative liver weights, but not absolute weights, for rats exposed to 1,000 ppm were significantly greater than those for controls. A mild nephropathy characterized by increased tubular casts was found in male rats exposed to 160, 400, and 1,000 ppm. No compound-related lesions were observed in female rats. Mean (± SE) male body weights (grams; *indicates statistically significant from controls) for 0, 25, 60, 160, 400, and 1,000 ppm were 373 (10.5), 375 (7.5), 372 (6.5), 353 (6.3), 346 (8.7), and 302 (9.8)*, respectively. Male liver weights (grams) were 14.8 (0.74), 16.4 (0.71), 16.0 (0.54), 15.5 (0.58), 15.1 (0.51), and 16.0 (0.54), respectively. Male liver weight/body weight ratios (mg/g) were 39.5 (1.66), 43.6 (1.37), 43.0 (1.29), 43.8 (1.27), 43.9 (1.51), and 52.9 (1.55)*, respectively. Mean (± SE) female body weights (grams; *indicates statistically significant from controls) for 0, 25, 60, 160, 400, and 1,000 ppm were 214 (4.9), 212 (4.3), 209 (3.3), 204 (1.2), 201 (2.2), and 189 (3.7)*, respectively. Female liver weights (grams) were 8.08 (0.48), 8.24 (0.42), 8.55 (0.30), 8.23 (0.31), 8.39 (0.52), and 9.12 (0.24), respectively. Female liver weight/body weight ratios (mg/g) were 38.0 (2.41), 38.8 (1.55), 41.0 (1.30), 40.4 (1.54), 41.7 (2.37), and 48.3 (1.51)*, respectively.

Conclusions

Based on the results of this study, a 2-year study was conducted using vinyl toluene concentrations of 0, 100, and 300 ppm.

Data Quality

Reliability: Acceptable

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Repeated Dose Toxicity – Study 3

Remarks: All key parameters (i.e., exposure conditions, number of animals, observations, etc.) were appropriate and adequately described in the study.

Reference

Toxicology and Carcinogenesis Studies of Vinyl Toluene (Mixed Isomers; 65-71% meta-isomer and 32-35% para-isomer) in F344/N Rats and B6C3F_1 Mice (Inhalation Studies). National Toxicology Program Technical Report Series No. 375, NIH Publication No. 90-2830.

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HUMAN HEALTH EFFECTS ELEMENTS
Repeated Dose Toxicity – Study 4

Test Substance

Remarks:	Vinyl Toluene (Mixed Isomers) Purity: Approximately 99% (combined 65%-71% meta-isomer and 32%-35% para-isomer) by gas chromatography Solvent carrier: None Contaminants: None of the potential degradation products was determined to be present at concentrations greater than 0.05% of the VT concentration. Chemical formula: C_9H_{10}

Method

Method/Guideline:	Study was performed under the direction of the NIEHS and conducted in compliance with National Toxicology Program (NTP) protocols. EPA OPPTS Method 870.3465 is a comparable method.
GLP:	Yes; reviewed and approved by the NTP Board of Scientific Counselor's Peer Review Panel.
Year of study:	1990
Species:	Mouse
Strain:	$B6C3F_1$
Sex:	Both
Number:	10 males and 10 females
Route of Exposure:	Inhalation
Exposure period:	13 weeks
Frequency:	6h/day for 64 d over 13 weeks
Doses:	0, 10, 25, 60, and 160 ppm
Control:	Yes
Post observation:	All surviving animals were sacrificed after 13 week exposure
Statistical Method:	ANOVA with Dunnett's test for multiple comparisons for organ weights.
Remarks:	Thirteen-week studies were conducted to evaluate the cumulative toxic effects of repeated inhalation of vinyl toluene and to determine the concentrations to be used in the 2-year study. Male and female $B6C3F_1$ mice (8 to 9 weeks old) were observed for 20 days and assigned to groups according to tables of random numbers. Feed was available *ad libitum* during nonexposure periods; water was available at all times. Groups of 10 mice of each sex were exposed to air concentrations of VT at target

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HUMAN HEALTH EFFECTS ELEMENTS
Repeated Dose Toxicity – Study 4

concentrations of 0, 10, 25, 60, and 160 ppm, 6 hours per day, 5 days per week for 13 weeks (64 exposures). VT vapor was generated by using either a J-tube or a gas dispersion-type system in which heated air was passed through liquid VT. The vapor then entered the airstream near the top of the chamber and was mixed in the chamber plenum before entering the exposure area of the chamber. The concentration of VT in the chambers and the exposure room was monitored by an automatic sampling system coupled to a gas chromatograph (Varian 2700) equipped with a flame ionization detector and a 3% SP2250 column. Uniformity of vapor concentration in each exposure chamber and at each position with animals present was measured periodically throughout the study by the same system used for daily concentration monitoring to validate the use of single-port sampling for daily concentration monitoring. The coefficients of variation were always found to be less than 10%.

Animals were observed one or two times per day; moribund animals were killed. Clinical signs were recorded once per week. Individual animal weights were recorded once per week. At the end of the 13-week studies, survivors were killed. A necropsy was performed on all animals except those excessively autolyzed or cannibalized. Histologic examinations were performed on 9 male mice and 10 female mice in the control groups, and all mice in the 25, 60, and 160 ppm groups. Tissues examined include: adrenal glands, bone, brain, cecum, colon, duodenum, epididymis/seminal vesicles/prostate/testes or oviduct/ovaries/uterus, esophagus, heart, ileum, jejunum, kidneys, larynx, liver, gallbladder, lungs, mammary gland, mandibular and mesenteric lymph nodes, mesentery, nasal passage, pancreas, parathyroid glands, pituitary gland, preputial or clitoral gland, rectum, salivary glands, skin, spinal cord, spleen, stomach, thymus, thyroid gland, trachea, and urinary bladder.

Results

NOAEL dose:	10 ppm
NOAEL effect:	Decrease in body weights.
LOAEL dose:	25 ppm

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HUMAN HEALTH EFFECTS ELEMENTS
Repeated Dose Toxicity – Study 4

LOAEL effect: Decrease in body weights.
Statistical results: Significant at $p<0.01$ for body weights (male and female).
Results Remarks: The incidence of deaths was not related to the exposure concentration. The final mean body weights of male mice exposed to 25, 60, and 160 ppm were 12, 12, and 20% lower, respectively, than controls. The final mean body weights of female mice exposed to 25, 60, and 160 ppm were 13, 14, and 16% lower, respectively, compared to controls. Lethargy was observed for mice exposed to 60 and 160 ppm; palpebral closure was observed for mice exposed to 160 ppm. The relative liver weights for exposed and control mice were not significantly different. Inflammation of the lung was observed in 5/10 male and 3/9 female mice exposed to 160 ppm, in 4/9 male and 2/10 female mice exposed to 60 ppm, and in 1/10 female controls. Metaplasia of the respiratory epithelium of the nasal turbinates (hyaline cytoplasmic alteration) was seen in all exposed groups. Acute inflammation and/or metaplasia of the nasal turbinates were seen in 7/10 male and 9/9 female mice exposed to 160 ppm, 7/8 male and 10/10 female mice exposed to 60 ppm, 8/9 male and 9/10 female mice exposed to 25 ppm, 3/10 male and 4/10 female mice exposed to 10 ppm, and 1/10 female controls. Lesions of the lungs and nasal turbinates were not seen in the male controls. Mean (± SE) male body weights (grams; *indicates statistically significant from controls) for 0, 10, 25, 60, and 160 ppm were 33.1 (0.70), 33.4 (1.12), 29.1 (0.53)*, 29.0 (0.41)*, and 26.5 (0.46)*, respectively. Male liver weights (grams) were 1.55 (0.21), 1.62 (0.064), 1.51 (0.056), 1.47 (0.062), and 1.21 (0.071, respectively. Male liver weight/body weight ratios (mg/g) were 46.5 (6.1), 48.7 (2.0), 52.0 (2.1), 50.7 (1.7), and 45.6 (2.8), respectively. Mean (± SE) female body weights (grams; *indicates statistically significant from controls) for 0, 10, 25, 60, and 160 ppm were 27.6 (0.79), 25.8 (0.56), 24 (0.39)*, 23.8 (0.36)*, and 23.2 (0.33)*, respectively. Female liver weights (grams) were 1.46 (0.094), 1.34 (0.074), 1.23 (0.045), 1.15 (0.078)*, and 1.09 (0.066)*, respectively. Female liver weight/body weight ratios (mg/g) were 52.9 (3.3), 52.2 (2.9), 51.4 (1.9), 48.3 (3.2), and 46.9 (2.8), respectively.

ROBUST SUMMARY
STYRENE, AR-METHYL-(VINYL TOLUENE)
CAS # 25013-15-4

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Conclusions

Because of lower mean body weight gain of animals exposed to 25 ppm or more, lesions in the nasal passage, and lethargy at 60 ppm and higher, inhalation exposure concentrations selected for mice for the 2-year study were 10 and 25 ppm.

Data Quality

Reliability: Acceptable

Remarks: All key parameters (i.e., exposure conditions, number of animals, observations, etc.) were appropriate and adequately described in the study.

Reference

Toxicology and Carcinogenesis Studies of Vinyl Toluene (Mixed Isomers; 65-71% meta-isomer and 32-35% para-isomer) in F344/N Rats and B6C3F_1 Mice (Inhalation Studies). National Toxicology Program Technical Report Series No. 375, NIH Publication No. 90-2830.

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Test Substance

Remarks:	Vinyl Toluene (Mixed Isomers) Purity: Approximately 99% (combined 65%-71% meta-isomer and 32%-35% para-isomer) by gas chromatography Solvent carrier: None Contaminants: None of the potential degradation products was determined to be present at concentrations greater than 0.05% of the VT concentration. Chemical formula: C_9H_{10}

Method

Method/Guideline:	Study was performed under the direction of the NIEHS and conducted in compliance with National Toxicology Program (NTP) protocols. EPA OPPTS Method 870.3465 is a comparable method.
GLP:	Yes; reviewed and approved by the NTP Board of Scientific Counselor's Peer Review Panel.
Year of study:	1990
Species:	Rat
Strain:	F344/N rats
Sex:	Both
Number:	49 or 50 males and 50 females
Route of Exposure:	Inhalation
Exposure period:	103 weeks
Frequency:	6h/day, 5 days/week for 103 weeks
Doses:	0, 100, and 300 ppm
Control:	Yes
Post observation:	All surviving animals were sacrificed after 103 week exposure
Statistical Method:	The probability of survival was estimated by the product-limit procedure of Kaplan and Meier (1958). Statistical analyses for a possible dose-related effect on survival used the method of Cox (1972) for testing two groups for equality and Tarone's (1975) life table test for a dose-related trend. The method used for analysis of tumor incidence was a logistic regression analysis. Alternative methods included the life table test, Fisher exact test and the Cochran-Armitage trend test. Test of significance include pairwise comparisons for each dosed group with controls and a test for an

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overall dose-response trend. Continuity-corrected tests were used in the analysis of tumor incidence, and reported P values are one-sided. The procedures described above also were used to evaluate selected nonneoplastic lesions.

Remarks: The purpose of this study was to evaluate the long-term toxicity, carcinogenicity, and neoplasm-promoting potential of VT in rats. Male and female F344/N rats (9 to 10 weeks old) were observed for 19 days, and assigned to groups according to tables of random numbers. Feed was available *ad libitum* during nonexposure periods; water was available at all times. Groups of 49 or 50 male rats and 50 female rats were exposed to air concentrations of VT at target concentrations of 0, 100, and 300 ppm, 6 hours per day, 5 days per week for 103 weeks. VT vapor was generated by using either a J-tube or a gas dispersion-type system in which heated air was passed through liquid VT. The vapor then entered the airstream near the top of the chamber and was mixed in the chamber plenum before entering the exposure area of the chamber. The concentration of VT in the chambers and the exposure room was monitored by an automatic sampling system coupled to a gas chromatograph (Varian 2700) equipped with a flame ionization detector and a 3% SP2250 column. Uniformity of vapor concentration in each exposure chamber and at each position with animals present was measured periodically throughout the studies by the same system used for daily concentration monitoring to validate the use of single-port sampling for daily concentration monitoring. The coefficients of variation were always found to be less than 10%.

All animals were observed two times per day. Individual body weights were recorded once per week for the first 13 weeks of the study and once per month thereafter. Mean body weights were calculated for each group. Animals found moribund and those surviving to the end of the study were sacrificed. A necropsy was performed on all animals, including those found dead. One male from the control group was found to have been missexed and was discarded. During necropsy, all organs and tissues were examined for grossly visible lesions. The following tissues were examined histologically for control and high dose groups and animals dying before month 20 and 21: adrenal glands, bone, brain, cecum, colon,

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duodenum, epididymis/seminal vesicles/prostate/testes or oviduct/ovaries/uterus, esophagus, heart, ileum, jejunum, kidneys, larynx, liver, lungs, mammary gland, mandibular and mesenteric lymph nodes, mesentery, nasal passage, pancreas, parathyroid glands, pituitary gland, preputial or clitoral gland, rectum, salivary glands, skin, spinal cord, spleen, stomach, thymus, thyroid gland, trachea, and urinary bladder. Lungs and nasal passage were examined for the low dose group.

Results

Results Remarks: Mean body weights of male rats exposed to 300 ppm and those of female rats exposed to 100 and 300 ppm were generally 4-11% lower than controls. No significant differences in survival were observed between any groups of rats of either sex (male: control 19/49; low dose, 17/50; high dose, 19/50; female: 31/50, 28/50, 26/50). Survival P values for males were as follows: controls, 0.762; 100 ppm, 0.825; 300 ppm, 0.878. Survival values for females were as follows: controls, 0.372; 100 ppm, 0.733, 300 ppm, 0.399. Degenerative and nonneoplastic proliferative lesions of the nasal mucosa were observed at increased incidences in exposed rats. These lesions included diffuse hyperplasia (goblet cell) of the respiratory epithelium with intraepithelial mucous cysts and focal erosion of the olfactory epithelium with cystic dilatation (cysts) of the Bowman's glands. Focal respiratory epithelial metaplasia of the olfactory epithelium was seen in some exposed males, and cells with homogeneous eosinophilic cytoplasm in the olfactory epithelium was seen in some exposed males, and cells with homogeneous eosinophilic cytoplasm in the olfactory epithelium occurred at increased incidences in exposed female rats. Neoplasms of the nasal mucosa were not seen in male or female rats. There were no chemically related increases in neoplasm incidence in exposed male or female rats. Mean male body weight (grams) for controls, 100, and 300 ppm was as follows: weeks 1-13: 294, 291, and 277, respectively; weeks 17-49: 414, 411, and 394, respectively; weeks 53-101: 454, 444, and 433, respectively. Mean female body weight (grams) for controls, 100, and 300 ppm was as follows: weeks 1-13: 179, 173, and 171, respectively; weeks 17-49: 241, 226, and 226, respectively; weeks 53-101: 319, 291, and 290, respectively. Degenerative and nonneoplastic

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proliferative lesions occurred at increased incidences in the olfactory and respiratory epithelium. In the olfactory epithelium of males, 6/50 exhibited cysts at 300 ppm, 8/50 exhibited erosion at 100 ppm, and 6/50 exhibited metaplasia at 100 ppm. In the male respiratory epithelium, 13/50 and 9/50 exhibited cysts in 100 and 300 ppm, respectively, and 24/50 and 28/50 exhibited hyperplasia in 100 and 300 ppm, respectively. In the olfactory epithelium of females, 5/49 and 13/50 exhibited cysts in 100 and 300 ppm, respectively, and 9/49 and 21/50 exhibited hyperplasia (and eosinophilic cytoplasm) in 100 and 300 ppm, respectively. In the respiratory epithelium of females, 6/49 and 10/50 exhibited cysts in 100 and 300 ppm, respectively, and 19/49 and 19/50 exhibited hyperplasia in 100 and 300 ppm, respectively.

Lipomas were observed in 2/50 male rats exposed to 300 ppm. The lipoma of the kidney is a benign mesenchymal neoplasm consisting of completely differentiated fat cells with interspersed fibrocytes, collagen, and blood vessels. The historical incidence of renal mesenchyme neoplasms in male F344/N rats is 0/346 for chamber controls and 2/1,590 (0.1%) for untreated controls; the highest observed incidence is 1/50.

Papillomas of the transitional epithelium were observed in 2/49 male rats exposed to 300 ppm. The historical incidence of urinary bladder transitional epithelium neoplasms in male F344/N rats is 3/339 (0.9%) for chamber controls and 1/1,552 (0.1%) for untreated controls; no more than one neoplasm has been observed in any control group. Hyperplasia of the transitional epithelium was seen in a single high concentration female rat.

Conclusions

The lowered body weights and the chemically related lesions occurring in the nasal passage indicated that the concentrations were adequate for assessing the long-term toxicity and carcinogenicity of VT and that higher doses would not have been appropriate.

The most striking change observed in the nasal passage was a loss of olfactory epithelium with replacement by respiratory epithelium. The Bowman’s gland beneath the olfactory epithelium also were

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often replaced by ciliated columnar cells similar to the respiratory epithelium. These changes may represent an adaptive response of the olfactory epithelium. There was a mild diffuse hyperplasia of the respiratory epithelium, which is often seen in association with an inflammatory response. Focal proliferative lesions or neoplasms of the nasal passage were not found. Transitional cell papillomas of the urinary bladder were seen in two male rats exposed to 300 ppm but were not considered to be related to VT exposure. Three mesenchymal renal neoplasms were found in the 300 ppm male rats. Two of these were diagnosed as lipomas and are considered spurious and unrelated to VT exposure. The third renal mesenchymal neoplasm was a sarcoma that contained marked osseous metaplasia. This neoplasm was considered unrelated to VT exposure.

No clinical or histologic evidence of nerve damage was seen after 2 years. Epoxide intermediates are possibly formed by the metabolism of VT (Heinonen and Vainio, 1980), and it has been suggested that the main reactive intermediate is vinyl toluene-7,8-oxide (Heinonen, 1984). Because of their electrophilic character, the intermediates could bind to nucleic acids and proteins, leading to toxicity, mutagenicity, and carcinogenicity. Although VT was mutagenic in cultured mammalian cells, there was no evidence of carcinogenicity in this study. The reason for the lack of carcinogenicity is unknown, but given the toxicity seen in the nasal passage and the body weight losses, it is unlikely that the rats could have tolerated much higher concentrations.

Under the conditions of the 2-year inhalation studies, there was no evidence of carcinogenic activity for male or female F344/N rats exposed to 100 or 300 ppm vinyl toluene.

Data Quality

Reliability: Acceptable

Remarks: All key parameters (i.e., exposure conditions, number of animals, observations, etc.) were appropriate and adequately described in the study.

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Reference

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Cox, D.R. (1972) Regression models and life tables. J.R. Stat. Soc. B34:187-220.

Heinonen, T. and Vainio, H. (1980) Vinyltoluene induced changes in xenobiotic-metabolizing enzyme activities and tissue glutathione content in various rodent species. Biochem. Pharmacol. 29:2675-2679.

Heinonen, T.H.H. (1984) Metabolism of vinyltoluene in the rat: Effect of induction and inhibition of the cytochrome P-450. Biochem. Pharmacol. 33:1585-1593.

Kaplan, E.L. and Meier, P. (1958) Nonparametric estimation from incomplete observations. J. Am. Stat. Assoc. 53:457-481.

Tarone, R.E. (1975) Tests for trend in life table analysis. Biometrika 62:679-682.

TABLE A5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS IN THE TWO-YEAR INHALATION STUDY OF VINYL TOLUENE (Continued)

	Chamber Control	100 ppm	300 ppm
GENITAL SYSTEM			
Epididymis	(47)	(16)	(50)
Dilatation, multifocal	2 (4%)		1 (2%)
Granuloma sperm, single	1 (2%)		1 (2%)
Inflammation, suppurative, focal	1 (2%)		
Left, atrophy			1 (2%)
Preputial gland	(46)	(18)	(48)
Abscess		2 (11%)	
Atrophy, diffuse	1 (2%)		
Ectasia	2 (4%)	2 (11%)	3 (6%)
Fibrosis, diffuse	2 (4%)		
Inflammation, chronic active	4 (9%)		4 (8%)
Inflammation, granulomatous, diffuse	1 (2%)		1 (2%)
Inflammation, granulomatous, focal	4 (9%)		3 (6%)
Inflammation, granulomatous, multifocal	18 (39%)	4 (22%)	22 (46%)
Inflammation, suppurative, focal	1 (2%)		
Prostate	(49)	(18)	(49)
Hyperplasia, focal	5 (10%)		5 (10%)
Hyperplasia, multifocal	3 (6%)	2 (11%)	6 (12%)
Inflammation, chronic	1 (2%)		
Inflammation, chronic active	8 (16%)	6 (33%)	13 (27%)
Seminal vesicle	(41)	(13)	(43)
Atrophy, diffuse	3 (7%)		5 (12%)
Fibrosis, multifocal			1 (2%)
Inflammation, subacute, diffuse	1 (2%)		
Inflammation, suppurative, chronic active, diffuse			1 (2%)
Testes	(49)	(42)	(50)
Hemorrhage		1 (2%)	
Mineralization, focal	1 (2%)		
Mineralization, multifocal	1 (2%)	1 (2%)	1 (2%)
Arteriole, inflammation	6 (12%)	2 (5%)	4 (8%)
Capsule, hyperplasia, multifocal			1 (2%)
Interstitial cell, hyperplasia	2 (4%)	4 (10%)	2 (4%)
Seminiferous tubule, atrophy	13 (27%)	11 (26%)	13 (26%)
Seminiferous tubule, degeneration	2 (4%)		4 (8%)
Serosa, necrosis, focal	1 (2%)	1 (2%)	2 (4%)
HEMATOPOIETIC SYSTEM			
Bone marrow	(48)	(15)	(50)
Atrophy		1 (7%)	
Hyperplasia	8 (17%)	2 (13%)	5 (10%)
Metaplasia, osseous	1 (2%)	1 (7%)	
Myelofibrosis	1 (2%)		
Myeloid cell, hyperplasia	4 (8%)		4 (8%)
Lymph node	(49)	(27)	(50)
Inflammation, suppurative, focal	1 (2%)		
Lumbar, hemorrhage			1 (2%)
Lumbar, inflammation, granulomatous, multifocal	1 (2%)		
Mediastinal, hemorrhage	2 (4%)	2 (7%)	5 (10%)
Mediastinal, hyperplasia, lymphoid	1 (2%)		
Mediastinal, hyperplasia, re cell	1 (2%)		
Mediastinal, pigmentation, hemosiderin	2 (4%)		
Pancreatic, hemorrhage		1 (4%)	
Pancreatic, hyperplasia, lymphoid	2 (4%)		
Lymph node, mandibular	(44)	(14)	(48)
Hemorrhage		1 (7%)	1 (2%)
Hyperplasia, lymphoid	2 (5%)		3 (6%)
Hyperplasia, plasma cell	2 (5%)	1 (7%)	5 (10%)
Hyperplasia, re cell	1 (2%)		
Inflammation, subacute, focal	1 (2%)		

TABLE B4. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS IN THE TWO-YEAR INHALATION STUDY OF VINYL TOLUENE (Continued)

	Chamber Control	100 ppm	300 ppm
ENDOCRINE SYSTEM			
Adrenal gland, cortex (Continued)	(50)	(14)	(50)
Hyperplasia, focal	9 (18%)		2 (4%)
Hyperplasia, multifocal		1 (7%)	1 (2%)
Hypertrophy, focal	4 (8%)		1 (2%)
Hypertrophy, multifocal	2 (4%)	1 (7%)	1 (2%)
Pigmentation, hemosiderin, diffuse	2 (4%)		1 (2%)
Pigmentation, hemosiderin, multifocal			1 (2%)
Adrenal gland, medulla	(48)	(14)	(45)
Angiectasis, multifocal	1 (2%)		1 (2%)
Hyperplasia, focal	5 (10%)	1 (7%)	5 (11%)
Hyperplasia, multifocal	2 (4%)		3 (7%)
Vacuolization cytoplasmic, diffuse	1 (2%)		
Islets, pancreatic	(50)	(12)	(49)
Ectopic tissue, focal	1 (2%)		
Hyperplasia, focal			4 (8%)
Hyperplasia, multifocal	2 (4%)		
Hypertrophy, focal	2 (4%)		1 (2%)
Hypoplasia, diffuse	1 (2%)		
Pituitary gland	(50)	(43)	(50)
Pars distalis, angiectasis, focal	4 (8%)	1 (2%)	2 (4%)
Pars distalis, angiectasis, multifocal	2 (4%)	3 (7%)	
Pars distalis, concretion			1 (2%)
Pars distalis, cyst	5 (10%)	10 (23%)	10 (20%)
Pars distalis, cyst, multiple		1 (2%)	
Pars distalis, hemorrhage, chronic, focal	1 (2%)		
Pars distalis, hyperplasia	1 (2%)	1 (2%)	1 (2%)
Pars distalis, hyperplasia, focal	3 (6%)	2 (5%)	2 (4%)
Pars distalis, hyperplasia, multifocal		1 (2%)	1 (2%)
Pars distalis, pigmentation, hemosiderin, diffuse	(2%)		
Pars intermedia, pigmentation, hemosiderin, diffuse	1 (2%)		
Pars nervosa, pigmentation, diffuse			1 (2%)
Thyroid gland	(50)	(10)	(50)
Cyst	2 (4%)		1 (2%)
Developmental malformation		(10%)	
C-cell, hyperplasia, focal	2 (4%)		3 (6%)
C-cell, hyperplasia, multifocal	21 (42%)	1 (10%)	7 (14%)
GENERAL BODY SYSTEM			
Tissue, NOS		(1)	
Necrosis, acute		1 (100%)	
GENITAL SYSTEM			
Clitoral gland	(48)	(11)	(46)
Abscess		1 (9%)	
Atrophy		1 (9%)	1 (2%)
Ectasia		2 (18%)	4 (9%)
Hyperplasia	3 (6%)	1 (9%)	3 (7%)
Inflammation, acute	2 (4%)		1 (2%)
Inflammation, chronic active	1 (2%)	1 (9%)	2 (4%)
Inflammation, granulomatous	2 (4%)		3 (7%)
Ovary	(50)	(18)	(50)
Angiectasis, multifocal	1 (2%)		
Congestion, diffuse	1 (2%)		1 (2%)
Cyst		5 (28%)	3 (6%)
Hyperplasia	1 (2%)		

TABLE B4. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS IN THE TWO-YEAR INHALATION STUDY OF VINYL TOLUENE (Continued)

	Chamber Control	100 ppm	300 ppm
GENITAL SYSTEM (Continued)			
Uterus	(50)	(18)	(50)
Adenomyosis, focal			1 (2%)
Cyst	1 (2%)		1 (2%)
Dilatation	1 (2%)	3 (17%)	2 (4%)
Inflammation, acute, multifocal	1 (2%)		
Inflammation, chronic active	1 (2%)		1 (2%)
Endometrium, hyperplasia, focal	1 (2%)		
Epithelium, hyperplasia, diffuse	1 (2%)		1 (2%)
Epithelium, hyperplasia, focal	2 (4%)		1 (2%)
Lumen, hemorrhage		1 (6%)	1 (2%)
HEMATOPOIETIC SYSTEM			
Bone marrow	(49)	(9)	(50)
Hyperplasia	2 (4%)		3 (6%)
Hyperplasia, megakaryocyte			1 (2%)
Metaplasia, osseous, diffuse	1 (2%)		
Myeloid cell, hyperplasia	6 (12%)		2 (4%)
Lymph node	(50)	(17)	(50)
Hyperplasia, re cell	1 (2%)		
Mediastinal, hemorrhage	4 (8%)		3 (6%)
Mediastinal, hyperplasia, re cell			1 (2%)
Mediastinal, pigmentation, hemosiderin, diffuse			1 (2%)
Pancreatic, hemorrhage	1 (2%)	1 (6%)	
Pancreatic, inflammation, acute		1 (6%)	
Pancreatic, pigmentation, hemosiderin			1 (2%)
Renal, hyperplasia, plasma cell			1 (2%)
Renal, hyperplasia, re cell			1 (2%)
Lymph node, mandibular	(47)	(11)	(47)
Edema			1 (2%)
Hyperplasia, lymphoid			1 (2%)
Hyperplasia, plasma cell	2 (4%)		5 (11%)
Hyperplasia, re cell		1 (9%)	1 (2%)
Artery, inflammation, chronic active, focal			1 (2%)
Lymph node, mesenteric	(47)	(12)	(46)
Hemorrhage	3 (6%)	3 (25%)	2 (4%)
Hyperplasia, plasma cell	1 (2%)		
Hyperplasia, re cell	4 (9%)		3 (7%)
Pigmentation, hemosiderin		1 (8%)	
Spleen	(50)	(19)	(50)
Fibrosis, focal		1 (5%)	2 (4%)
Fibrosis, multifocal			1 (2%)
Granuloma, multifocal	1 (2%)		
Hematopoietic cell proliferation	5 (10%)		5 (10%)
Hyperplasia, reticulum cell, multifocal	1 (2%)		
Metaplasia, osseous, focal		1 (5%)	
Pigmentation, hemosiderin	11 (22%)	1 (5%)	15 (30%)
Capsule, ectopic tissue	1 (2%)		
Capsule, infiltration cellular, lymphocytic, multifocal	1 (2%)		
Thymus	(38)	(6)	(37)
Ectopic parathyroid gland	1 (3%)	2 (33%)	
Hyperplasia, tubular, diffuse	6 (16%)		14 (38%)
Infiltration cellular, polymorphonuclear	1 (3%)		
Artery, mediastinum, inflammation, chronic active, multifocal	1 (3%)		

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Test Substance

Remarks:	Vinyl Toluene (Mixed Isomers) Purity: Approximately 99% (combined 65%-71% meta-isomer and 32%-35% para-isomer) by gas chromatography Solvent carrier: None Contaminants: None of the potential degradation products was determined to be present at concentrations greater than 0.05% of the VT concentration. Chemical formula: C_9H_{10}

Method

Method/Guideline:	Study was performed under the direction of the NIEHS and conducted in compliance with National Toxicology Program (NTP) protocols. EPA OPPTS Method 870.3465 is a comparable method.
GLP:	Yes; reviewed and approved by the NTP Board of Scientific Counselor's Peer Review Panel.
Year of study:	1990
Species:	Mouse
Strain:	B6C3F_1 mice
Sex:	Both
Number:	50 males and 50 females
Route of Exposure:	Inhalation
Exposure period:	103 weeks
Frequency:	6h/day, 5 days/week for 103 weeks
Doses:	0, 10, and 25 ppm
Control:	Yes
Post observation:	All surviving animals were sacrificed after 103 week exposure
Statistical Method:	The probability of survival was estimated by the product-limit procedure of Kaplan and Meier (1958). Statistical analyses for a possible dose-related effect on survival used the method of Cox (1972) for testing two groups for equality and Tarone's (1975) life table test for a dose-related trend. The method used for analysis of tumor incidence was a logistic regression analysis. Alternative methods included the life table test, Fisher exact test and the Cochran-Armitage trend test. Test of significance include pairwise comparisons for each dosed group with controls and a test for an

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overall dose-response trend. Continuity-corrected tests were used in the analysis of tumor incidence, and reported P values are one-sided. The procedures described above also were used to evaluate selected nonneoplastic lesions.

Remarks: The purpose of this study was to evaluate the long-term toxicity, carcinogenicity, and neoplasm-promoting potential of VT in mice. Male and female B6C3F_1 mice (8 to 9 weeks old) were observed for 19 days, and assigned to groups according to tables of random numbers. Feed was available *ad libitum* during nonexposure periods; water was available at all times. Groups of 50 male and female rats were exposed to air concentrations of VT at target concentrations of 0, 10, and 25 ppm, 6 hours per day, 5 days per week for 103 weeks. VT vapor was generated by using either a J-tube or a gas dispersion-type system in which heated air was passed through liquid VT. The vapor then entered the airstream near the top of the chamber and was mixed in the chamber plenum before entering the exposure area of the chamber. The concentration of VT in the chambers and the exposure room was monitored by an automatic sampling system coupled to a gas chromatograph (Varian 2700) equipped with a flame ionization detector and a 3% SP2250 column. Uniformity of vapor concentration in each exposure chamber and at each position with animals present was measured periodically throughout the studies by the same system used for daily concentration monitoring to validate the use of single-port sampling for daily concentration monitoring. The coefficients of variation were always found to be less than 10%.

All animals were observed two times per day. Individual body weights were recorded once per week for the first 13 weeks of the study and once per month thereafter. Mean body weights were calculated for each group. Animals found moribund and those surviving to the end of the study were sacrificed. A necropsy was performed on all animals, including those found dead. During necropsy, all organs and tissues were examined for grossly visible lesions. The following tissues were examined histologically for control and high dose groups and animals dying before month 20 and 21: adrenal glands, bone, brain, cecum, colon, duodenum, epididymis/seminal vesicles/prostate/testes or

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oviduct/ovaries/uterus, esophagus, heart, ileum, jejunum, kidneys, larynx, liver, gallbladder, lungs, mammary gland, mandibular and mesenteric lymph nodes, mesentery, nasal passage, pancreas, parathyroid glands, pituitary gland, preputial or clitoral gland, rectum, salivary glands, skin, spinal cord, spleen, stomach, thymus, thyroid gland, trachea, and urinary bladder. Lungs and nasal passage were examined for the low dose group.

Results

Results Remarks: Males surviving to end of the study was as follows: controls, 33/50; 10 ppm, 30/50; 25 ppm, 41/50. Females surviving to the end of the study was as follows: controls, 36/50; 10 ppm, 37/50; 25 ppm, 34/50. Survival P values (the result of the life table trend for chamber controls and the results of the life table pairwise comparisons with the chamber controls for the dose groups) for males are as follows: controls, 0.026; 10 ppm, 0.472; 25 ppm, 0.031. Survival P values for females are as follows: controls, 0.434; 10 ppm, 0.847; 25 ppm, 0.473. Mean body weights of mice exposed to 25 ppm were 10-23% lower than those of controls after week 8; mean body weights of mice exposed to 10 ppm were 5-14% lower than those of controls after week 37 for males and week 41 for females. No compound-related clinical signs were observed. During week 21, liquid vinyl toluene entered the 25 ppm chamber due to a technical error in the connection of the vinyl toluene vapor generation lines; two males and six females came into direct contact with the liquid and died or were killed in a moribund condition. The survival of male mice exposed to 25 ppm was significantly greater than that of controls after day 707. No other significant differences in survival were seen between any groups of either sex.

Increased incidences of chronic active inflammation and hyperplasia of the respiratory epithelium occurred in exposed mice. Lesions were located in the middle and posterior portions of the dorsal meatus. The severity of the lesions was dose related; inflammation and hyperplasia were generally mild and moderate in the 10-ppm males and females, respectively, and moderate and marked in the 25-ppm males and females, respectively. Inflammation was characterized by focal infiltration of the mucosa

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by varying numbers of neutrophils and mononuclear cells. Hyperplasia consisted of increased height of the epithelium, downgrowth of ciliated columnar cells into the submucosal glands, formation of intraepithelial glandlike structures, and extension of the respiratory epithelium into areas usually covered by olfactory epithelium. Neoplasms of the nasal passage were not observed.

Chronic active inflammation of the bronchioles occurred at increased incidences in exposed mice. The severity of the lesion was minimal to moderate and varied considerably within and among the exposure groups. It consisted of focal accumulation of neutrophils, macrophages, and lymphocytes within the walls of bronchioles and the interstitium of adjacent alveoli. The epithelium of affected alveoli had increased numbers of cuboidal cells; the lumina contained some inflammatory cells, proteinaceous material, and when severe, eosinophilic crystals and cholesterol clefts.

Alveolar/bronchiolar neoplasms occurred with a significant negative trend in male mice, and the incidences in the 25-ppm group were significantly lower than those in controls.

Lymphomas in males occurred with a significant negative trend; the incidence in the group exposed to 25 ppm was significantly lower than that in the controls. A marginal ($p<0.10$) decrease was also observed for exposed females (chamber control, 16/48; low dose, 9/49; high dose, 8/50).

The incidences of hepatocellular carcinomas and adenomas or carcinomas (combined) in the female group exposed to 25 ppm were significantly lower than those in controls.

Mean male body weight (grams) for controls, 10, and 25 ppm was as follows: weeks 1-13: 29.3, 29.4, and 26.6, respectively; weeks 17-49: 36.0, 34.3, and 30.2, respectively; weeks 53-101: 40.2, 37.1, and 33.0, respectively. Mean female body weight (grams) for controls, 10, and 25 ppm was as follows: weeks 1-13: 24.3 24.0, and 22.0, respectively; weeks 17-49: 30.0, 28.5, and 25.5, respectively; weeks 53-101: 34.6, 30.5, and 27.5, respectively.

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Numbers of mice with selected respiratory tract lesions was as follows: In the nasal passage of males, 47/48 and 48/49 exhibited chronic active inflammation in 10 and 25 ppm, respectively, 48/48 and 49/49 exhibited respiratory epithelium hyperplasia in 10 and 25 ppm, respectively, and 15/48 and 30/49 exhibited chronic active inflammation in the lung/bronchiole region in 10 and 25 ppm, respectively. In the nasal passage of females, 49/49 and 47/48 exhibited chronic active inflammation in 10 and 25 ppm, respectively, 49/49 and 47/48 exhibited respiratory epithelium hyperplasia in 10 and 25 ppm, respectively, and 14/49 and 37/49 exhibited chronic active inflammation in the lung/bronchiole region in 10 and 25 ppm, respectively.

The overall alveolar/bronchiolar adenoma or carcinoma rates for male mice were as follows: control, 12/50 (24%); 10 ppm, 5/49 (10%); 25 ppm, 2/49 (4%). The overall hepatocellular adenoma or carcinoma rates for female mice were as follows: control, 9/48 (19%); 10 ppm, 5/16 (31%); 25 ppm, 2/49 (4%). The overall rates of lymphoma in male mice were as follows: control, 7/50 (14%); 10 ppm, 3/50 (6%); 25 ppm 0/50 (0%).

Conclusions

Mice were approximately 10 times more sensitive to inhalation of vinyl toluene than were rats. The exact mechanisms that account for the species difference are not known.

During most of the 2-year study, the body weights of mice exposed to 25 ppm averaged 10-23% lower than those of controls, indicating that a higher exposure concentration could not have been tolerated even though there was no decreased survival in the exposed animals.

The lesions of the nasal passage in mice were analogous to those found in rats, with extension of respiratory epithelium into areas normally covered by olfactory epithelium. This may represent metaplasia of olfactory epithelium to ciliated cells similar in appearance to respiratory epithelium or loss of olfactory epithelium, with extension of the respiratory epithelium into areas of olfactory epithelium. This change was diagnosed as respiratory epithelial hyperplasia. As in the rats, ciliated cells extended down

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into and replaced the cells of the Bowman's glands. There also appeared to be some atrophy of the nerves in the submucosa of the nasal passage, a lesion not recognized in rats. No primary neoplasms were seen in the nasal passage in mice.

Negative trends in the incidences of neoplasms were associated with vinyl toluene exposure in mice. Malignant lymphomas showed a marked decline in males and a marginally significant decrease in females. Pulmonary neoplasms were markedly decreased in males, but no difference was seen in females. Female mice also showed a marginal decline in hepatocellular neoplasms. The mice in these studies showed weight reduction associated with vinyl toluene exposure that may have been due to toxicity or reduced food consumption.

Epoxide intermediates are possibly formed by the metabolism of VT (Heinonen and Vainio, 1980), and it has been suggested that the main reactive intermediate is vinyl toluene-7,8-oxide (Heinonen, 1984). Because of their electrophilic character, the intermediates could bind to nucleic acids and proteins, leading to toxicity, mutagenicity, and carcinogenicity. Although VT was mutagenic in cultured mammalian cells, there was no evidence of carcinogenicity in this study. The reason for the lack of carcinogenicity is unknown, but given the toxicity seen in the nasal passage and the body weight losses, it is unlikely that the mice could have tolerated much higher concentrations.

Under the conditions of the 2-year inhalation studies, there was no evidence of carcinogenic activity for male or female B6C3F_1 mice exposed to 10 or 25 ppm vinyl toluene.

Data Quality

Reliability: Acceptable

Remarks: All key parameters (i.e., exposure conditions, number of animals, observations, etc.) were appropriate and adequately described in the study.

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Reference

Toxicology and Carcinogenesis Studies of Vinyl Toluene (Mixed Isomers; 65-71% meta-isomer and 32-35% para-isomer) in F344/N Rats and B6C3F_1 Mice (Inhalation Studies). National Toxicology Program Technical Report Series No. 375, NIH Publication No. 90-2830.

Cox, D.R. (1972) Regression models and life tables. J.R. Stat. Soc. B34:187-220.

Heinonen, T. and Vainio, H. (1980) Vinyltoluene induced changes in xenobiotic-metabolizing enzyme activities and tissue glutathione content in various rodent species. Biochem. Pharmacol. 29:2675-2679.

Heinonen, T.H.H. (1984) Metabolism of vinyltoluene in the rat: Effect of induction and inhibition of the cytochrome P-450. Biochem. Pharmacol. 33:1585-1593.

Kaplan, E.L. and Meier, P. (1958) Nonparametric estimation from incomplete observations. J. Am. Stat. Assoc. 53:457-481.

Tarone, R.E. (1975) Tests for trend in life table analysis. Biometrika 62:679-682.

TABLE C5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE IN THE TWO-YEAR INHALATION STUDY OF VINYL TOLUENE (Continued)

	Chamber Control	10 ppm	25 ppm
CARDIOVASCULAR SYSTEM			
Heart	(50)	(13)	(50)
Cardiomyopathy		1 (8%)	1 (2%)
Artery, embolus, single	1 (2%)		
Artery, inflammation, chronic active		1 (8%)	
Coronary artery, infiltration cellular, mixed cell, focal	1 (2%)		
Mitral valve, pigmentation, hemosiderin, focal	1 (2%)		
Myocardium, inflammation, chronic active, multifocal	1 (2%)		
Perivascular, inflammation, acute, focal	1 (2%)		
Ventricle, karyomegaly, multifocal	1 (2%)		
Ventricle left, bacterium	1 (2%)		
Ventricle left, inflammation, subacute, focal	1 (2%)		
ENDOCRINE SYSTEM			
Adrenal gland	(49)	(10)	(48)
Accessory adrenal cortical nodule	1 (2%)		3 (6%)
Capsule, hyperplasia, focal	3 (6%)		2 (4%)
Capsule, hyperplasia, multifocal	36 (73%)	3 (30%)	39 (81%)
Adrenal gland, cortex	(49)	(10)	(48)
Hyperplasia, focal	5 (10%)		7 (15%)
Hyperplasia, multifocal	6 (12%)		5 (10%)
Hypertrophy, focal	7 (14%)	1 (10%)	8 (17%)
Hypertrophy, multifocal	6 (12%)		3 (6%)
Adrenal gland, medulla	(49)	(9)	(47)
Hyperplasia, focal	1 (2%)		
Islets, pancreatic	(49)	(9)	(49)
Hyperplasia, focal			2 (4%)
Hyperplasia, multifocal	7 (14%)	1 (11%)	4 (8%)
Parathyroid gland	(36)	(6)	(35)
Infiltration cellular, lymphocytic, focal			1 (3%)
Pituitary gland	(45)	(11)	(47)
Pars distalis, cyst	2 (4%)		2 (4%)
Thyroid gland	(49)	(9)	(47)
Infiltration cellular, lymphocytic, focal			1 (2%)
Inflammation, chronic, focal			1 (2%)
Ultimobranchial cyst	1 (2%)		2 (4%)
Follicular cell, hyperplasia	1 (2%)		
Follicular cell, hyperplasia, multifocal	1 (2%)		
GENERAL BODY SYSTEM			
Tissue, NOS		(1)	(1)
Inflammation, chronic active, multifocal		1 (100%)	
GENITAL SYSTEM			
Coagulating gland	(1)		
Inflammation, chronic active	1 (100%)		
Preputial gland	(15)	(6)	(8)
Abscess	6 (40%)	1 (17%)	
Cyst	5 (33%)	1 (17%)	8 (100%)
Cyst multilocular	1 (7%)		
Dilatation		1 (17%)	
Inflammation, chronic	3 (20%)	3 (50%)	
Inflammation, chronic active	7 (47%)		
Prostate	(46)	(9)	(44)
Infiltration cellular, lymphocytic, multifocal	1 (2%)		
Inflammation, chronic active	3 (7%)	1 (11%)	
Inflammation, suppurative, acute	1 (2%)		

TABLE C5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE IN THE TWO-YEAR INHALATION STUDY OF VINYL TOLUENE (Continued)

	Chamber Control	10 ppm	25 ppm
GENITAL SYSTEM (Continued)			
Seminal vesicle	(48)	(17)	
Dilatation	1 (2%)	1 (6%)	
Testes	(50)	(12)	(49)
Left, hemorrhage			1 (2%)
Seminiferous tubule, atrophy	3 (6%)	1 (8%)	3 (6%)
Seminiferous tubule, degeneration, multifocal	1 (2%)		
HEMATOPOIETIC SYSTEM			
Bone marrow	(49)	(10)	(49)
Myeloid cell, hyperplasia	5 (10%)	2 (20%)	
Lymph node	(50)	(26)	
Angiectasis, focal		1 (4%)	
Bronchial, hemorrhage			1 (2%)
Inguinal, hyperplasia, lymphoid		1 (4%)	
Inguinal, hyperplasia, re cell	1 (2%)		
Lumbar, hemorrhage	1 (2%)		
Lumbar, hyperplasia, lymphoid	1 (2%)		
Mediastinal, hyperplasia, lymphoid		1 (4%)	1 (2%)
Lymph node, mandibular	(47)	(11)	(35)
Hyperplasia, lymphoid			2 (6%)
Hyperplasia, re cell	3 (6%)		3 (9%)
Lymph node, mesenteric	(42)	(19)	(33)
Hematopoietic cell proliferation	6 (14%)	4 (21%)	
Hemorrhage	25 (60%)	12 (63%)	14 (42%)
Hyperplasia		1 (5%)	
Hyperplasia, lymphoid		1 (5%)	2 (6%)
Hyperplasia, re cell	2 (5%)		3 (9%)
Spleen	(49)	(14)	(49)
Angiectasis, multifocal			1 (2%)
Depletion lymphoid	1 (2%)		
Hematopoietic cell proliferation	11 (22%)	6 (43%)	2 (4%)
Hyperplasia, lymphoid	1 (2%)		1 (2%)
Hyperplasia, lymphoid, diffuse			1 (2%)
Hyperplasia, plasma cell			1 (2%)
Hyperplasia, re cell	1 (2%)		
Thrombus			1 (2%)
Lymphocyte, necrosis, multifocal			1 (2%)
Thymus	(35)	(6)	(34)
Cyst, multiple			1 (3%)
Depletion lymphoid	1 (3%)	(17%)	1 (3%)
Hyperplasia, lymphoid, diffuse			1 (3%)
Inflammation, acute		1 (17%)	
Syncytial alteration	1 (3%)		
INTEGUMENTARY SYSTEM			
Skin	(50)	(30)	(49)
Inflammation, chronic active	1 (2%)	1 (3%)	1 (2%)
Ulcer		1 (3%)	
Prepuce, abscess		2 (7%)	
Prepuce, inflammation, chronic active		1 (3%)	
Prepuce, necrosis	3 (6%)		
Prepuce, ulcer	2 (4%)	2 (7%)	
Scrotal, pigmentation, melanin, focal		1 (3%)	
Scrotal, hair follicle, hyperplasia, focal		1 (3%)	
Subcutaneous tissue, abscess		1 (3%)	
Subcutaneous tissue, cyst	1 (2%)		
Subcutaneous tissue, edema		1 (3%)	
Subcutaneous tissue, inflammation, granulomatous, focal	1 (2%)		

TABLE D5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE IN THE TWO-YEAR INHALATION STUDY OF VINYL TOLUENE (Continued)

	Chamber Control	10 ppm	25 ppm
GENERAL BODY SYSTEM			
None			
GENITAL SYSTEM			
Clitoral gland	(1)		(1)
Cyst, multifocal			1 (100%)
Ovary	(46)	(14)	(44)
Abscess, multiple	1 (2%)	1 (7%)	
Angiectasis			1 (2%)
Atrophy	1 (2%)		
Cyst	9 (20%)	9 (64%)	4 (9%)
Hemorrhage, focal	2 (4%)		
Hyperplasia, diffuse			1 (2%)
Infiltration cellular, lymphocytic	2 (4%)	1 (7%)	
Thrombus	1 (2%)		
Periovarian tissue, infiltration cellular, lymphocytic	4 (9%)		
Uterus	(48)	(37)	(45)
Abscess, single	1 (2%)		
Adenomyosis	1 (2%)		
Angiectasis	1 (2%)		
Dilatation	2 (4%)	5 (14%)	12 (27%)
Hemorrhage, acute	1 (2%)		
Hyperplasia, cystic	36 (75%)	29 (78%)	29 (64%)
Inflammation, chronic, diffuse			1 (2%)
Inflammation, chronic active, multifocal	1 (2%)		
Metaplasia, squamous			1 (2%)
Thrombus	1 (2%)		
Serosa, cyst	1 (2%)		
HEMATOPOIETIC SYSTEM			
Bone marrow	(47)	(2)	(47)
Hypoplasia, focal			1 (2%)
Myeloid cell, hyperplasia	1 (2%)		
Lymph node	(48)	(8)	(49)
Hyperplasia, lymphoid	1 (2%)		
Bronchial, depletion lymphoid, diffuse	1 (2%)		
Bronchial, hyperplasia, lymphoid			2 (4%)
Bronchial, hyperplasia, plasma cell	1 (2%)		1 (2%)
Mediastinal, hemorrhage	1 (2%)		
Mediastinal, hyperplasia, lymphoid			2 (4%)
Mediastinal, hyperplasia, plasma cell	1 (2%)		
Lymph node, mandibular	(45)	(1)	(43)
Hyperplasia, lymphoid	4 (9%)		3 (7%)
Hyperplasia, plasma cell	3 (7%)		
Hyperplasia, re cell	1 (2%)		1 (2%)
Infiltration cellular, polymorphonuclear, diffuse	1 (2%)		
Pigmentation, hemosiderin, diffuse	1 (2%)		2 (5%)
Lymph node, mesenteric	(38)	(6)	(32)
Hematopoietic cell proliferation		2 (33%)	
Hemorrhage	3 (8%)	3 (50%)	
Hyperplasia, lymphoid	4 (11%)		
Hyperplasia, plasma cell	1 (3%)		
Hyperplasia, re cell	1 (3%)		
Spleen	(47)	(13)	(49)
Angiectasis	1 (2%)		
Congestion			1 (2%)
Depletion lymphoid			2 (4%)
Developmental malformation			1 (2%)

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HUMAN HEALTH EFFECTS ELEMENTS
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Test Substance

Remarks: Vinyl Toluene (Mixed Isomers)
Purity: Approximately 98% (combined 55%-70% meta-isomer and 30%-45% para-isomer) obtained by ultraviolet or infrared analysis
Solvent carrier: None
Contaminants: None reported
Chemical formula: C_9H_{10}

Method

Method/Guideline:	Not referenced
GLP:	Unknown
Year of study:	1956
Species:	Rat
Strain:	Wistar
Sex:	Both
Number per dose:	10 to 25
Vehicle:	None
Route of Administration:	Inhalation
Concentrations:	580, 1130, and 1350 ppm
Duration:	Seven to eight hours/day, five days/week, for 139 days

Remarks: Male and female Wistar rats were used in repeated inhalation studies. The test substance was introduced into the exposure chamber by vaporizing into the in-flowing air at a constant rate a measured amount of the test substance. The vapor concentration within the exposure chamber (580, 1130, and 1350 ppm) was established using a sensitive analytical procedure and adjusted to the desired level, when possible, with the animals in the chamber and the chamber fan going to provide adequate atmospheric circulation. Analysis during the experiments demonstrated that the vapor concentrations were held uniformly within 10% of the desired concentration.

Two types of chambers were used. The large rectangular chambers were made of sheet metal and had a capacity of about 1700 liters and were equipped with a 30 inch circulating fan. The smaller chambers were fashioned of glass and were of about 160 liter capacity. Matched groups of 10 to 25 male and female rats

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were used. "Air-exposed" and/or "unexposed" controls were used. "Air-exposed" controls were subjected to repeated exposures of room air in a chamber, while the "unexposed" controls were maintained in a routine manner in the animal quarters. Routinely, the "air-exposed" and test animals were placed in the exposure chambers seven to eight hours daily, five days a week, for 139 days.

All animals were weighed at regular intervals during the study. Frequent observations were made of their general appearance and behavior. Records were kept of food consumption and mortality. Growth curves were drawn for each group. Those animals failing in health were killed when moribund and examined. Surviving animals were killed 18 to 22 hours after their last exposure and examined for evidence of organic injury. The gross appearance of the lungs, heart, liver, kidneys, spleen, and testes was observed, and these organs were weighed. In addition, portions of the adrenals, pancreas, and femoral bone marrow, as well as those tissues mentioned above, were preserved in formalin and saved for preparation of hematoxylin-and-eosin-stained sections. Oxalated blood was obtained from selected animals of each group at time of autopsy for determination of urea nitrogen by the diacetyl monoxime method. Bone marrow counts were made on selected animals of each group. The number of nucleated cells per cubic millimeter of femoral bone marrow was estimated by the method of Farrar (1936) using a red blood cell diluting pipette and 1% acetic acid as the diluting fluid. The total cell count was determined in a similar manner using Hayem's solution.

When applicable, the Fisher t-test was used in comparing the mean values of the final body and organ weights obtained on the exposed and unexposed animals. Probability values (P) of less than 0.05 were interpreted as indicating a significant difference. The criteria considered in judging the toxic effects on the test animals were growth, mortality, appearance and behavior, hematological findings, terminal concentration of urea nitrogen in the blood, final average organ and body weights, histopathological findings, and bone marrow counts.

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Results

NOAEL:	580 ppm
NOAEL effect:	Growth depression, increase in liver weights and liver histopathology
LOAEL:	1,130 ppm
LOAEL effect:	Growth depression, increase in liver weights and liver histopathology
Remarks:	No effects were seen at 580 ppm. At 1130 and 1350 ppm, liver histopathology characterized by fatty degeneration in the midzonal and central cells of the liver lobule was observed. A moderate degree of growth depression and increase in liver weight was also observed at 1130 and 1350 ppm. A moderate amount of mortality was seen in the 1350 ppm exposed animals.

Conclusions: The NOAEL was 580 ppm for rats exposed by inhalation of vinyl toluene (7-8 hours/day, 5 days/week, for 139 days). This effect was based on growth depression, increase in liver weights and liver histopathology.

Data Quality: Valid with restrictions

Remarks: Several experimental results (numbers dead, actual body weight change) were not fully reported.

Reference:

Wolf, M.A., Rowe, V.K., McCollister, D.D., Hollingsworth, R.L., and Oyen, F. (1956) Toxicological studies of certain alkylated benzenes and benzene. Am. Med. Assoc. Arch. Ind. Health 14:387-398.

Farrar, G.E., Jr. 1936 Concentration of nucleated cells in bone marrow of the albino rat. Am. J. Physiol. 117:662-664.

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CAS # 25013-15-4

HUMAN HEALTH EFFECTS ELEMENTS
Repeated Dose Toxicity – Study 8

Test Substance

Remarks: Vinyl Toluene (Mixed Isomers)
Purity: Approximately 98% (combined 55%-70% meta-isomer and 30%-45% para-isomer) obtained by ultraviolet or infrared analysis
Solvent carrier: None
Contaminants: None reported
Chemical formula: C_9H_{10}

Method

Method/Guideline:	Not referenced
GLP:	Unknown
Year of study:	1956
Species:	Albino guinea pigs
Strain:	Heterogeneous stock raised in the laboratory
Sex:	Both
Number per dose:	5 to 10
Vehicle:	None
Route of Administration:	Inhalation
Concentrations:	580, 1130, and 1350 ppm
Duration:	Seven to eight hours/day, five days/week, for 139 days

Remarks: Male and female albino guinea pigs were used in repeated inhalation studies. The test substance was introduced into the exposure chamber by vaporizing into the in-flowing air at a constant rate a measured amount of the test substance. The vapor concentration within the exposure chamber (580, 1130, and 1350 ppm) was established using a sensitive analytical procedure and adjusted to the desired level, when possible, with the animals in the chamber and the chamber fan going to provide adequate atmospheric circulation. Analysis during the experiments demonstrated that the vapor concentrations were held uniformly within 10% of the desired concentration.

Two types of chambers were used. The large rectangular chambers were made of sheet metal and had a capacity of about 1700 liters and were equipped with a 30 inch circulating fan. The smaller chambers were fashioned of glass and were of about 160 liter capacity. Matched groups of 5 to 10 male and female albino

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HUMAN HEALTH EFFECTS ELEMENTS
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guinea pigs were used. "Air-exposed" and/or "unexposed" controls were used. "Air-exposed" controls were subjected to repeated exposures of room air in a chamber, while the "unexposed" controls were maintained in a routine manner in the animal quarters. Routinely, the "air-exposed" and test animals were placed in the exposure chambers seven to eight hours daily, five days a week, for 139 days.

All animals were weighed at regular intervals during the study. Frequent observations were made of their general appearance and behavior. Records were kept of food consumption and mortality. Growth curves were drawn for each group. Those animals failing in health were killed when moribund and examined. Surviving animals were killed 18 to 22 hours after their last exposure and examined for evidence of organic injury. The gross appearance of the lungs, heart, liver, kidneys, spleen, and testes was observed, and these organs were weighed. In addition, portions of the adrenals, pancreas, and femoral bone marrow, as well as those tissues mentioned above, were preserved in formalin and saved for preparation of hematoxylin-and-eosin-stained sections. Oxalated blood was obtained from selected animals of each group at time of autopsy for determination of urea nitrogen by the diacetyl monoxime method. Bone marrow counts were made on selected animals of each group. The number of nucleated cells per cubic millimeter of femoral bone marrow was estimated by the method of Farrar (1936) using a red blood cell diluting pipette and 1% acetic acid as the diluting fluid. The total cell count was determined in a similar manner using Hayem's solution.

When applicable, the Fisher t-test was used in comparing the mean values of the final body and organ weights obtained on the exposed and unexposed animals. Probability values (P) of less than 0.05 were interpreted as indicating a significant difference. The criteria considered in judging the toxic effects on the test animals were growth, mortality, appearance and behavior, hematological findings, terminal concentration of urea nitrogen in the blood, final average organ and body weights, histopathological findings, and bone marrow counts.

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HUMAN HEALTH EFFECTS ELEMENTS
Repeated Dose Toxicity – Study 8

Results

NOAEL:	580 ppm
NOAEL effect:	Growth depression, increase in kidney weights and liver histopathology
LOAEL:	1,130 ppm
LOAEL effect:	Growth depression, increase in kidney weights and liver histopathology
Remarks:	No effects were seen at 580 ppm. At 1130 and 1350 ppm, liver histopathology characterized by fatty degeneration in the midzonal and central cells of the liver lobule was observed. A slight degree of growth depression and increase in kidney weight was also observed at 1130 and 1350 ppm. In addition, a slight increase in liver weights was observed at 1350 ppm.

Conclusions: The NOAEL was 580 ppm for guinea pigs exposed by inhalation of vinyl toluene (7-8 hours/day, 5 days/week, for 139 days). This effect was based on growth depression, increase in kidney weights and liver histopathology.

Data Quality: Valid with restrictions

Remarks: Several experimental results (actual body weight change and organ weights) were not fully reported.

Reference:

Wolf, M.A., Rowe, V.K., McCollister, D.D., Hollingsworth, R.L., and Oyen, F. (1956) Toxicological studies of certain alkylated benzenes and benzene. Am. Med. Assoc. Arch. Ind. Health 14:387-398.

Farrar, G.E., Jr. 1936 Concentration of nucleated cells in bone marrow of the albino rat. Am. J. Physiol. 117:662-664.

ROBUST SUMMARY
STYRENE, AR-METHYL-(VINYL TOLUENE)
CAS # 25013-15-4

HUMAN HEALTH EFFECTS ELEMENTS
Repeated Dose Toxicity – Study 9

Test Substance

Remarks: Vinyl Toluene (Mixed Isomers)
Purity: Approximately 98% (combined 55%-70% meta-isomer and 30%-45% para-isomer) obtained by ultraviolet or infrared analysis
Solvent carrier: None
Contaminants: None reported
Chemical formula: C_9H_{10}

Method

Method/Guideline:	Not referenced
GLP:	Unknown
Year of study:	1956
Species:	White rabbits
Strain:	Heterogeneous stock raised in the laboratory
Sex:	Both
Number per dose:	1 to 2
Vehicle:	None
Route of Administration:	Inhalation
Concentrations:	580, 1130, and 1350 ppm
Duration:	Seven to eight hours/day, five days/week, for 139 days

Remarks: Male and female white rabbits were used in repeated inhalation studies. The test substance was introduced into the exposure chamber by vaporizing into the in-flowing air at a constant rate a measured amount of the test substance. The vapor concentration within the exposure chamber (580, 1130, and 1350 ppm) was established using a sensitive analytical procedure and adjusted to the desired level, when possible, with the animals in the chamber and the chamber fan going to provide adequate atmospheric circulation. Analysis during the experiments demonstrated that the vapor concentrations were held uniformly within 10% of the desired concentration.

Two types of chambers were used. The large rectangular chambers were made of sheet metal and had a capacity of about 1700 liters and were equipped with a 30 inch circulating fan. The smaller chambers were fashioned of glass and were of about 160 liter capacity. Matched groups of 1 to 2 male and female rabbits

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were used. "Air-exposed" and/or "unexposed" controls were used. "Air-exposed" controls were subjected to repeated exposures of room air in a chamber, while the "unexposed" controls were maintained in a routine manner in the animal quarters. Routinely, the "air-exposed" and test animals were placed in the exposure chambers seven to eight hours daily, five days a week, for 139 days.

All animals were weighed at regular intervals during the study. Frequent observations were made of their general appearance and behavior. Records were kept of food consumption and mortality. Growth curves were drawn for each group. Those animals failing in health were killed when moribund and examined. Surviving animals were killed 18 to 22 hours after their last exposure and examined for evidence of organic injury. The gross appearance of the lungs, heart, liver, kidneys, spleen, and testes was observed, and these organs were weighed. In addition, portions of the adrenals, pancreas, and femoral bone marrow, as well as those tissues mentioned above, were preserved in formalin and saved for preparation of hematoxylin-and-eosin-stained sections. Oxalated blood was obtained from selected animals of each group at time of autopsy for determination of urea nitrogen by the diacetyl monoxime method. Bone marrow counts were made on selected animals of each group. The number of nucleated cells per cubic millimeter of femoral bone marrow was estimated by the method of Farrar (1936) using a red blood cell diluting pipette and 1% acetic acid as the diluting fluid. The total cell count was determined in a similar manner using Hayem's solution.

When applicable, the Fisher t-test was used in comparing the mean values of the final body and organ weights obtained on the exposed and unexposed animals. Probability values (P) of less than 0.05 were interpreted as indicating a significant difference. The criteria considered in judging the toxic effects on the test animals were growth, mortality, appearance and behavior, hematological findings, terminal concentration of urea nitrogen in the blood, final average organ and body weights, histopathological findings, and bone marrow counts.

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Results

NOAEL:	580 ppm
NOAEL effect:	Slight increase in kidney weights
LOAEL:	1,130 ppm
LOAEL effect:	Slight increase in kidney weights
Remarks:	No effects were seen at 580 ppm. At 1130 and 1350 ppm, a slight increase in kidney weights was observed. In addition, at 1350 ppm, liver histopathology which was characterized by fatty degeneration in the midzonal and central cells of the liver lobule was observed.

Conclusions: The NOAEL was 580 ppm for white rabbits exposed by inhalation of vinyl toluene (7-8 hours/day, 5 days/week, for 139 days). This effect was based a slight increase in kidney weights.

Data Quality: Valid with restrictions

Remarks: Several experimental results (actual body weight change and organ weights) were not fully reported.

Reference:

Wolf, M.A., Rowe, V.K., McCollister, D.D., Hollingsworth, R.L., and Oyen, F. (1956) Toxicological studies of certain alkylated benzenes and benzene. Am. Med. Assoc. Arch. Ind. Health 14:387-398.

Farrar, G.E., Jr. 1936 Concentration of nucleated cells in bone marrow of the albino rat. Am. J. Physiol. 117:662-664.

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Test Substance

Remarks:	Vinyl Toluene (Mixed Isomers) Purity: Approximately 98% (combined 55%-70% meta-isomer and 30%-45% para-isomer) obtained by ultraviolet or infrared analysis Solvent carrier: None Contaminants: None reported Chemical formula: C_9H_{10}

Method

Method/Guideline:	Not referenced
GLP:	Unknown
Year of study:	1956
Species:	Rhesus monkeys
Strain:	Imported animals
Sex:	Both
Number per dose:	1 to 2
Vehicle:	None
Route of Administration:	Inhalation
Concentrations:	580, 1130, and 1350 ppm
Duration:	Seven to eight hours/day, five days/week, for 139 days
Remarks:	Male and female Rhesus monkeys were used in repeated inhalation studies. The test substance was introduced into the exposure chamber by vaporizing into the in-flowing air at a constant rate a measured amount of the test substance. The vapor concentration within the exposure chamber (580, 1130, and 1350 ppm) was established using a sensitive analytical procedure and adjusted to the desired level, when possible, with the animals in the chamber and the chamber fan going to provide adequate atmospheric circulation. Analysis during the experiments demonstrated that the vapor concentrations were held uniformly within 10% of the desired concentration. The large rectangular chambers were made of sheet metal and had a capacity of about 1700 liters and were equipped with a 30 inch circulating fan. Matched groups of 1 to 2 male and female Rhesus monkeys were used. "Air-exposed" and/or "unexposed" controls were used. "Air-exposed" controls were subjected to repeated exposures of room air in a chamber, while the "unexposed"

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controls were maintained in a routine manner in the animal quarters. Routinely, the "air-exposed" and test animals were placed in the exposure chambers seven to eight hours daily, five days a week, for 139 days.

All animals were weighed at regular intervals during the study. Frequent observations were made of their general appearance and behavior. Records were kept of food consumption and mortality. Growth curves were drawn for each group. Those animals failing in health were killed when moribund and examined. Surviving animals were killed 18 to 22 hours after their last exposure and examined for evidence of organic injury. The gross appearance of the lungs, heart, liver, kidneys, spleen, and testes was observed, and these organs were weighed. In addition, portions of the adrenals, pancreas, and femoral bone marrow, as well as those tissues mentioned above, were preserved in formalin and saved for preparation of hematoxylin-and-eosin-stained sections. Oxalated blood was obtained from selected animals of each group at time of autopsy for determination of urea nitrogen by the diacetyl monoxime method. Bone marrow counts were made on selected animals of each group. The number of nucleated cells per cubic millimeter of femoral bone marrow was estimated by the method of Farrar (1936) using a red blood cell diluting pipette and 1% acetic acid as the diluting fluid. The total cell count was determined in a similar manner using Hayem's solution.

When applicable, the Fisher t-test was used in comparing the mean values of the final body and organ weights obtained on the exposed and unexposed animals. Probability values (P) of less than 0.05 were interpreted as indicating a significant difference. The criteria considered in judging the toxic effects on the test animals were growth, mortality, appearance and behavior, hematological findings, terminal concentration of urea nitrogen in the blood, final average organ and body weights, histopathological findings, and bone marrow counts.

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Results

NOAEL:	≥1,350 ppm, the highest dose tested
NOAEL effect:	No effects were observed
LOAEL:	>1,350 ppm, the highest dose tested
LOAEL effect:	No effects were observed
Remarks:	No effects at any exposure level were observed.

Conclusions: The NOAEL was ≥1,350 ppm for Rhesus monkeys exposed by inhalation of vinyl toluene (7-8 hours/day, 5 days/week, for 139 days). No effects were observed at any dose level.

Data Quality: Valid with restrictions

Remarks: Several experimental results (actual body weight change and organ weights) were not fully reported.

Reference:

Wolf, M.A., Rowe, V.K., McCollister, D.D., Hollingsworth, R.L., and Oyen, F. (1956) Toxicological studies of certain alkylated benzenes and benzene. Am. Med. Assoc. Arch. Ind. Health 14:387-398.

Farrar, G.E., Jr. 1936 Concentration of nucleated cells in bone marrow of the albino rat. Am. J. Physiol. 117:662-664.

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Developmental Toxicity/Teratogenicity – Study 1

Test Substance

Remarks:	Methyl styrene (isomer % not referenced) Purity: Not referenced Solvent carrier: Corn oil Contaminants: None reported Chemical formula: C_9H_{10}

Method

Method/Guideline:	Not referenced
GLP:	Not indicated
Year of study:	1981
Species:	Rats
Strain:	Sprague-Dawley
Sex:	Female
Number per dose:	10-15
Route of Administration:	Intraperitoneal
Days of Gestation:	1-15
Concentrations:	250 mg/kg (MTD)
Duration:	Once daily through day 15 gestation
Control Group:	Yes
Statistical Method:	Fisher's exact test
Remarks:	Probe studies were conducted on rats injected intraperitoneally with the test chemical dissolved or suspended in corn oil. Initial dose-response studies with nonpregnant rats established the maximum tolerated dose (MTD), defined as the dose at which there was no mortality, no marked signs of toxicity (such as unconsciousness), and less than a 10% reduction (relative to controls) in body weight gain during or within two weeks following the course of 15 daily IP injections. After the MTD determination, young adult female Sprague-Dawley rats (250-300 g) were caged with breeder males of the same strain. The females were examined daily for the presence of sperm in a vaginal lavage. The day sperm was detected was designated as day 1 of gestation. Inseminated females were randomly assigned to treatment and control groups. Treatment began on day 1 of gestation and continued through day 15. Controls were injected intraperitoneally with corn oil, while the test group received the test chemical in corn oil at the MTD (240 mg/kg). Each group was composed of

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10-15 inseminated females individually housed in stainless steel wire mesh cages with free access to food and water.

On day 21 of gestation, the females were killed by decapitation and the uterine contents were examined. The individual fetuses were weighed, measured for crown-rump length, sexed, and examined for externally visible malformations. One-half to two-thirds of each litter was preserved in Bouin's fluid for internal examination by the Wilson method of free-hand razor-blade sectioning, and the balance of each litter was preserved in ethanol for clearing and skeletal staining with alizarin red. The internal organs of the maternal rats were examined grossly, and the brain, heart, lungs, liver, spleen, kidneys, adrenals, and ovaries were weighed and then preserved in 10% formalin for histopathological examination.

Results:

Remarks: The incidence of resorptions was significantly increased ($p<0.05$), and the fetal sex ratio was significantly altered ($p<0.05$) with a deficit of female fetuses. No teratogenic effects were observed. In addition, no treatment-related histopathological changes were observed in maternal tissues. Detailed results such as body weights, incidence of resoprtions, fetal sex ratio, histopathological changes, etc. were not reported.

Conclusions: Treatment with the MTD (250 mg/kg) daily during days 1-15 of gestation in the rat did not produce any teratogenic effects. However the incidence of resorptions was significantly increased ($p<0.05$), and the fetal sex ratio was significantly altered ($p<0.05$) with a deficit of female fetuses.

Data Quality:

Reliability: Acceptable with restrictions

Remarks: No information about the test compound was given except that it was methyl styrene. This study has been referenced in summary

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data of vinyl toluene, but no information as to purity and isomer composition was reported. In addition, summary data tables of the body weights, fetal toxicity data or maternal histopathology was not reported.

Reference: Hardin, B.D., Bond, G.P., Sikov, M.R., Andrew, F.D., Beliles, R.P., and Niemeier, R.W. 1981. Testing of selected workplace chemicals for teratogenic potential. Scand. J. Work Environ. Health, 7(4):66-75

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Toxicity in Vitro (Gene Mutations) – Study 1

Test Substance

Remarks:	Vinyl Toluene (Mixed Isomers) Purity: Approximately 99% (combined 65%-71% meta-isomer and 32%-35% para-isomer) by gas chromatography Solvent carrier: Dimethyl sulfoxide Contaminants: None of the potential degradation products was determined to be present at concentrations greater than 0.05% of the VT concentration. Chemical formula: C_9H_{10}

Method

Method/Guideline:	Study was performed under the direction of the NIEHS and conducted in compliance with National Toxicology Program (NTP) protocols. EPA OPPTS Method 870.5100 is a comparable method.
GLP:	Yes; reviewed and approved by the NTP Board of Scientific Counselor's Peer Review Panel.
Year of study:	1990
Test Type:	*Salmonella typhimurium* reverse mutation assay
System of testing:	Bacterial
Species:	*Salmonella typhimurium*
Metabolic Activation:	A 9,000 x g supernatant from Aroclor 1254-induced male Sprague Dawley rat or Syrian hamster liver
Concentration:	0, 1, 3.3, 10, 33, 100, 333, and 1,000 ìg/plate
Statistical Method:	None referenced
Remarks:	Testing was performed as reported by Ames *et al*. (1975) with modifications as described in Zeiger *et al*. (1987) and Mortelmans *et al*. (1986). The study chemical was incubated with the *Salmonella typhimurium* tester strains (TA98, TA100, TA1535, and TA1537) either in buffer or S9 mix (metabolic activation enzymes and cofactors from Aroclor 1254-induced male Sprague Dawley rat or Syrian hamster liver) for 20 minutes at 37ºC before the addition of soft agar supplemented with L-histidine and D-biotin and subsequent plating on minimal glucose agar plates. Incubation was continued for an additional 48 hours. Chemicals were tested in four strains. Each test consisted of triplicate plates of concurrent positive and negative controls and of at least five doses of the study chemical. A positive control, 2-aminoanthracene was used on all strains in the presence of S9. In

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the absence of metabolic activation, 4-nitro-*o*-phenylenediamine was used with TA98, sodium azide was used with TA100 and TA1535, and 9-aminoacridine was used with TA1537. The high dose was limited by toxicity or solubility but did not exceed 1 mg/plate. All negative assays were repeated.

A positive response was defined as a reproducible, dose-related increase in histidine-independent (revertant) colonies in any one strain/activation combination. An equivocal response was defined as an increase in revertants that was not dose related, not reproducible, or of insufficient magnitude to support a determination of mutagenicity. A response was considered negative when no increase in revertant colonies was observed after chemical treatment.

Results

Result: Negative

Cytotoxic

Concentration: The test substance was toxic to all strains at 1,000 ìg/plate with or without metabolic activation. The dose of 333 ìg/plate was toxic to TA100 and TA1537 (with metabolic activation, rat S9 only).

Remarks: Vinyl toluene did induce gene mutations in *S. typhimurium* strains TA98, TA100, TA1535, or TA1537 when tested in a preincubation protocol at doses up to 1,000 ìg/plate with or without Aroclor 1254-induced male Sprague Dawley rat or Syrian hamster liver S9. The mean number of revertants/plate are presented in Table H1.

Conclusions Vinyl toluene did induce gene mutations in *S. typhimurium* strains TA98, TA100, TA1535, or TA1537 with or without exogenous metabolic activation (S9).

Data Quality

Reliability: Reliable

Remarks: The key parameters (i.e., dose levels, strains, use of positive controls) were appropriate and adequately described.

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Reference

Toxicology and Carcinogenesis Studies of Vinyl Toluene (Mixed Isomers; 65-71% meta-isomer and 32-35% para-isomer) in F344/N Rats and B6C3F_1 Mice (Inhalation Studies). National Toxicology Program Technical Report Series No. 375, NIH Publication No. 90-2830.

Ames, B.N., McCann, J. and Yamasaki, E. (1975) Methods for detecting carcinogens and mutagens with the *Salmonella*/mammalian-microsome mutagenicity test. Mutat. Res. 31:347-364.

Mortelmans, K., Haworth, S., Lawlor, T., Speck, W., Tainer, B. and Zeiger, E. (1986) *Salmonella* mutagenicity tests. II. Results from the testing of 270 chemicals. Environ. Mutagen. 8(Suppl. 7):1-119.

Zeiger, E., Anderson, B., Haworth, S., Lawlor, T., Mortelmans, K. and Speck, W. (1987) *Salmonella* mutagenicity tests. III. Results from the testing of 255 chemicals. Environ. Mutagen. 9(Suppl. 9): 1-110.

TABLE H1. MUTAGENICITY OF VINYL TOLUENE IN *SALMONELLA TYPHIMURIUM* (a)

Strain	Dose (µg/plate)	Revertants/Plate (b) −S9 Trial 1	−S9 Trial 2	+10% S9 (hamster) Trial 1	+10% S9 (hamster) Trial 2	+10% S9 (rat) Trial 1	+10% S9 (rat) Trial 2
TA100	0	151 ± 10.0	137 ± 7.4	216 ± 9.3	140 ± 7.5	214 ± 9.7	146 ± 8.1
	1	--	--	--	--	--	129 ± 5.7
	3.3	--	119 ± 4.7	216 ± 4.4	167 ± 7.9	190 ± 7.8	124 ± 4.7
	10	169 ± 3.2	132 ± 12.7	213 ± 7.1	170 ± 9.5	202 ± 10.1	130 ± 4.0
	33	163 ± 7.1	122 ± 3.2	216 ± 23.8	171 ± 3.2	227 ± 12.0	141 ± 5.8
	100	157 ± 25.9	132 ± 1.7	222 ± 3.3	168 ± 11.5	220 ± 11.7	128 ± 4.1
	333	165 ± 12.5	127 ± 10.1	189 ± 8.8	172 ± 6.0	Toxic	
	1,000	Toxic					
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control (c)		1,423 ± 77.5	1,254 ± 31.9	1,680 ± 64.7	2,507 ± 173.1	2,719 ± 231.9	2,265 ± 36.3
TA1535	0	17 ± 0.6	8 ± 1.8	18 ± 3.6	13 ± 0.3	19 ± 1.2	10 ± 1.2
	3.3	--	8 ± 0.0	24 ± 3.2	9 ± 1.0	25 ± 0.3	11 ± 2.0
	10	15 ± 2.4	8 ± 1.3	16 ± 2.6	11 ± 2.0	25 ± 2.1	10 ± 3.1
	33	19 ± 0.9	7 ± 1.0	20 ± 0.6	12 ± 2.6	14 ± 4.9	10 ± 2.5
	100	17 ± 4.0	7 ± 1.5	15 ± 1.2	10 ± 1.8	18 ± 4.2	11 ± 1.5
	333	1 ± 0.9	2 ± 0.7	18 ± 4.0	12 ± 1.7	29 ± 1.0	10 ± 2.3
	1,000	0 ± 0.0					
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control (c)		699 ± 72.5	642 ± 9.8	406 ± 53.2	253 ± 16.7	278 ± 44.0	209 ± 7.0
TA1537	0	11 ± 3.5	5 ± 1.0	15 ± 0.9	9 ± 2.3	15 ± 1.8	11 ± 1.3
		--	--	--	--	6 ± 0.9	--
	3.3	--	5 ± 0.3	11 ± 0.7	9 ± 1.2	12 ± 2.3	6 ± 0.6
	10	18 ± 0.3	4 ± 1.0	15 ± 1.5	6 ± 1.8	16 ± 0.9	8 ± 0.9
	33	15 ± 0.9	3 ± 0.3	11 ± 1.2	8 ± 1.5	12 ± 2.7	5 ± 1.3
	100	11 ± 2.3	4 ± 1.2	15 ± 1.2	6 ± 0.9	14 ± 2.3	7 ± 1.0
	333	2 ± 0.7	2 ± 0.3	10 ± 2.7	8 ± 2.0	Toxic	
	1,000	0 ± 0.0					
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control (c)		465 ± 44.5	253 ± 68.4	173 ± 10.0	226 ± 55.7	211 ± 8.8	185 ± 39.6
TA98	0	19 ± 1.7	13 ± 2.9	38 ± 3.3	17 ± 0.9	30 ± 5.7	24 ± 2.6
	3.3	--	9 ± 1.9	30 ± 5.7	17 ± 1.2	32 ± 4.9	18 ± 1.5
	10	16 ± 3.0	8 ± 0.0	45 ± 2.7	16 ± 2.5	40 ± 2.2	14 ± 0.9
	33	24 ± 2.4	10 ± 1.5	40 ± 3.0	17 ± 3.2	35 ± 3.0	10 ± 2.0
	100	22 ± 2.5	9 ± 1.5	43 ± 1.5	12 ± 0.7	36 ± 4.3	17 ± 4.0
	333	16 ± 1.3	8 ± 1.2	34 ± 4.2	9 ± 0.9	26 ± 2.8	6 ± 1.0
	1,000	Toxic					
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control (c)		182 ± 24.9	357 ± 36.5	553 ± 64.6	1,542 ± 45.0	391 ± 44.1	1,545 ± 112.3

(a) Study performed at Case Western Reserve University. The detailed protocol is presented in Zeiger et al. (1987). Cells and study compound or solvent (dimethyl sulfoxide) were incubated in the absence of exogenous metabolic activation (−S9) or with Aroclor 1254-induced S9 from male Syrian hamster liver or male Sprague Dawley rat liver. High dose was limited by toxicity or solubility but did not exceed 10 mg/plate; 0 µg/plate dose is the solvent control.
(b) Revertants are presented as mean ± standard error from three plates.
(c) Positive control; 2-aminoanthracene was used on all strains in the presence of S9. In the absence of metabolic activation, 4-nitro-*o*-phenylenediamine was used with TA98, sodium azide was used with TA100 and TA1535, and 9-aminoacridine was used with TA1537.

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Test Substance

Remarks:	Vinyl Toluene (Mixed Isomers) Purity: Approximately 99% (combined 65%-71% meta-isomer and 32%-35% para-isomer) by gas chromatography Solvent carrier: Dimethyl sulfoxide Contaminants: None of the potential degradation products was determined to be present at concentrations greater than 0.05% of the VT concentration. Chemical formula: C_9H_{10}

Method

Method/Guideline:	Study was performed under the direction of the NIEHS and conducted in compliance with National Toxicology Program (NTP) protocols. EPA OPPTS Method 870.5300 is a comparable method.
GLP:	Yes; reviewed and approved by the NTP Board of Scientific Counselor's Peer Review Panel.
Year of study:	1990
Test Type:	Mouse lymphoma assay
System of testing:	Non-bacterial
Species:	Mouse L5178Y/TK lymphoma cells
Metabolic Activation:	A 9,000 x g supernatant from Aroclor 1254-induced male F344 rats
Concentration:	Trial 1: 12.5, 25, 50, and 100 ìg/ml; Trial 2: 10, 20, 40, 60, and 80 ìg/ml; Trial 3: 40, 45, 50, 55, 60, and 65 ìg/ml
Statistical Method:	All data were evaluated statistically for both trend and peak response ($p<0.05$ for at least one of the three highest dose sets). Both responses must be significantly ($p<0.05$) positive for a chemical to be considered capable of inducing trifluorothymidine (Tf) resistance. If only one of these responses is significant, the call is "equivocal"; the absence of both trend and peak response results in a "negative" call.
Remarks:	The experimental protocol is presented in detail by McGregor *et al*. (1988) and follows the basic format of Clive *et al*. (1979). The highest dose of the study compound was determined by solubility or toxicity and did not exceed 100 ìg/ml. Mouse L5178Y/TK lymphoma cells were maintained at 37ºC as suspension cultures in Fischer's medium supplemented with 2 mM L-glutamine,

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100 ìg/ml sodium pyruvate, 0.05% pluronic F68, antibiotics, and heat-inactivated horse serum; normal cycling time was about 10 hours. To reduce the number of spontaneously occurring trifluorothymidine (Tft)-resistant cells, subcultures were exposed once to medium containing thymidine, hypoxanthine, methotrexate, and glycine for 1 day, to thymidine, hypoxanthine, and glycine for 1 day, and to normal medium for 3-5 days. For cloning, horse serum content was increased and Noble agar was added. Freshly prepared S9 metabolic activation factors were obtained from the liver of either Aroclor 1254-induced or noninduced male F344 rats.

All dose within an experiment, including concurrent positive and solvent controls, were replicated. Treated cultures contained 6 x 10^6 cells in 10 ml of medium. Incubation with the study chemical continued for 4 hours, after which time the medium plus chemical was removed and the cells were resuspended in 20 ml of fresh medium and incubated for an additional 2 days to express the mutant phenotype. Cell density was monitored so that log phase growth was maintained. After the 48-hour expression period, 3 x 10^6 cells were plated in medium and soft agar supplemented with Tft for selection of Tft-resistant cells ($TK^{+/+}$), and 600 cells were plated in nonselective medium and soft agar to determine cloning efficiency. Plates were incubated a 37ºC under 5% carbon dioxide for 10-12 days. All data were evaluated statistically for both trend and peak response. Both responses had to be significant ($p<0.05$) for a chemical to be considered capable of inducing Tft resistance; a single significant response led to an "equivocal" conclusion, and the absence of both a trend and a peak response resulted in a "negative" call. Minimum criteria for accepting an experiment as valid and a detailed description of the statistical analysis and data evaluation are presented in Myhr *et al*. (1985). This assay was performed without S9.

Results Positive without metabolic activation

Cytotoxic
Concentration: 65, 80, and 100 ìg/ml

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Remarks: In the mouse lymphoma assay for induction of Tft resistance in L5178Y cells, vinyl toluene gave a positive response in two trials conducted without S9 at the highest doses tested; these doses also produced severe toxicity, as evidenced by a relative total growth of less than 10% (McGregor *et al*., 1988; Table H2).

Conclusions: Vinyl toluene was positive in the mouse lymphoma assay for induction of trifluorothymidine resistance in L5178Y/TK cells in the absence of S9; it was not tested with S9.

Data Quality
Reliability: Acceptable
Remarks: The key parameters (i.e., concentrations, use of positive controls) were appropriate and adequately described.

Reference:

Toxicology and Carcinogenesis Studies of Vinyl Toluene (Mixed Isomers; 65-71% meta-isomer and 32-35% para-isomer) in F344/N Rats and B6C3F_1 Mice (Inhalation Studies). National Toxicology Program Technical Report Series No. 375, NIH Publication No. 90-2830.

Clive, D., Johnson, K.O., Spector, J.F.S., Batson, A.G. and Brown, M.M.M. (1979) Validation and characterization of the L5178Y/$TK^{+/-}$ mouse lymphoma mutagen assay system. Mutat. Res. 59:61-108.

McGregor, D.B., Brown, A., Cattanach, P., Edwards, I., McBride, D., Riach, C. and Caspary, W.J. (1988) Responses of the L5178Y tk+/tk- mouse lymphoma cell forward mutation assay: III. 72 coded chemicals. Environ. Molec. Mutagen 12:85-154.

Myhr, B., Bowers, L. and Caspary, W.J. (1985) Assays for the induction of gene mutations at the thymidine kinase locus in L5178Y mouse lymphoma cells in culture. Prog. Mutat. Res. 5:555-568.

TABLE H2. INDUCTION OF TRIFLUOROTHYMIDINE RESISTANCE BY VINYL TOLUENE IN MOUSE L5178Y/TK LYMPHOMA CELLS (a,b)

Compound	Concentration (μg/ml)	Cloning Efficiency (percent)	Relative Total Growth (percent)	Tft-Resistant Cells	Mutant Fraction (c)
Trial 1					
Dimethyl sulfoxide		80.3 ± 11.3	100.3 ± 13.0	96.8 ± 10.8	42.8 ± 7.0
Vinyl toluene	12.5	54.0 ± 1.0	71.0 ± 13.0	88.0 ± 13.0	54.0 ± 7.0
	25	70.0 ± 3.0	88.5 ± 0.5	99.5 ± 11.5	47.5 ± 3.5
	50	70.5 ± 0.5	69.0 ± 7.0	123.5 ± 14.5	58.5 ± 6.5
	100	Lethal			
Methyl methanesulfonate	15	27.0 ± 4.0	22.0 ± 5.0	559.5 ± 33.5	(d) 708.0 ± 145.0
Trial 2					
Dimethyl sulfoxide (e)		65.5 ± 2.1	100.0 ± 3.6	137.5 ± 5.7	70.5 ± 4.6
Vinyl toluene	10	65.0 ± 8.0	100.0 ± 9.0	120.5 ± 9.5	62.0 ± 3.0
	(f) 20	68.3 ± 3.4	66.0 ± 3.5	134.7 ± 7.5	66.3 ± 5.3
	(f) 40	60.7 ± 0.9	34.7 ± 5.5	185.7 ± 10.5	102.0 ± 5.0
	60	45.5 ± 5.5	5.5 ± 0.5	417.0 ± 2.0	(d) 311.0 ± 40.0
	(f) 80	Lethal	--	--	
Methyl methanesulfonate	15	23.0 ± 2.0	19.5 ± 0.5	255.0 ± 8.0	(d) 378.5 ± 49.5
Trial 3					
Dimethyl sulfoxide (f)		68.3 ± 7.9	100.0 ± 6.8	110.7 ± 17.5	54.0 ± 2.3
Vinyl toluene	(f) 40	58.3 ± 6.2	65.0 ± 7.1	47.3 ± 6.2	28.3 ± 6.4
	(f) 45	62.0 ± 6.6	55.7 ± 5.5	62.7 ± 9.8	33.3 ± 2.2
	(f) 50	65.7 ± 5.7	35.7 ± 2.9	61.7 ± 8.1	31.3 ± 2.2
	(f) 55	69.3 ± 11.4	29.3 ± 4.4	79.0 ± 13.9	38.3 ± 3.2
	(f) 60	54.3 ± 5.8	8.0 ± 1.0	240.3 ± 54.8	(d) 146.3 ± 29.6
	(f) 65	Lethal	--	--	--
Methyl methanesulfonate	15	20.5 ± 3.5	17.0 ± 3.0	165.0 ± 20.0	(d) 283.5 ± 79.5

(a) Study performed at Inveresk Research International. The experimental protocol is presented in detail by McGregor et al. (1988) and follows the basic format of Clive et al. (1979). The highest dose of study compound is determined by solubility or toxicity and may not exceed 5 mg/ml. All doses are tested in duplicate, unless otherwise indicated; the average for the tests is presented in the table. Cells (6×10^5/ml) were treated for 4 hours at 37° C in medium, washed, resuspended in medium, and incubated for 48 hours at 37° C. After expression, 3×10^6 cells were plated in medium and soft agar supplemented with trifluorothymidine (Tft) for selection of Tft-resistant cells, and 600 cells were plated in nonselective medium and soft agar to determine the cloning efficiency.

(b) Mean ± standard error from replicate trials of approximately 1×10^6 cells each. All data are evaluated statistically for both trend and peak response ($P<0.05$ for at least one of the three highest dose sets). Both responses must be significantly ($P<0.05$) positive for a chemical to be considered capable of inducing Tft resistance. If only one of these responses is significant, the call is "equivocal"; the absence of both trend and peak response results in a "negative" call.

(c) Mutant fraction (frequency) is a ratio of the Tft-resistant cells to the cloning efficiency, divided by 3 (to arrive at MF per 1×10^6 cells treated); MF = mutant fraction.

(d) Significant positive response; occurs when the relative mutant fraction (average MF of treated culture/average MF of solvent control) is greater than or equal to 1.6.

(e) Data presented are the results of four tests.

(f) Data presented are the results of three tests.

ROBUST SUMMARY
STYRENE, AR-METHYL-(VINYL TOLUENE)
CAS # 25013-15-4

HUMAN HEALTH EFFECTS ELEMENTS
Toxicity in Vitro (Gene Mutations) – Study 3

Test Substance

Remarks:	Vinyl Toluene (Mixed Isomers) Purity: Approximately 99% (combined 65%-71% meta-isomer and 32%-35% para-isomer) by gas chromatography Solvent carrier: Dimethyl sulfoxide Contaminants: None of the potential degradation products was determined to be present at concentrations greater than 0.05% of the VT concentration. Chemical formula: C_9H_{10}

Method

Method/Guideline:	Study was performed under the direction of the NIEHS and conducted in compliance with National Toxicology Program (NTP) protocols. EPA OPPTS Method 870.5375 and 870.5900 are comparable methods.
GLP:	Yes; reviewed and approved by the NTP Board of Scientific Counselor's Peer Review Panel.
Year of study:	1990
Test Type:	Sister chromatid exchange assay and chromosomal aberrations
Species:	Chinese hamster ovary (CHO) cells
Metabolic Activation:	A 9,000 x g supernatant from Aroclor 1254-induced male Sprague Dawley rat liver
Concentrations:	Sister chromatid exchange Without metabolic activation - Trial 1: 1.6, 5, 16, and 50 ìg/ml; Trial 2: 5, 10, 25, 50, 75, 100, and 150 ìg/ml; Trial 3: 25, 50, 75, and 100 ìg/ml. With metabolic activation – Trial 1: 5, 16, and 50 ìg/ml; Trial 2: 10, 25, 50, and 75 ìg/ml. Chromosomal aberrations Without metabolic activation: 1.6, 5, 16, and 50 ìg/ml. With metabolic activation: 5, 16, and 50 ìg/ml.
Remarks:	Testing was performed as reported by Galloway *et al*. (1987). Chemicals were tested in cultured Chinese hamster ovary (CHO) cells for induction of sister chromatid exchanges (SCEs) and chromosomal aberrations both in the presence and absence of Aroclor 1254-induced male Sprague Dawley rat liver S9 and cofactor mix. Cultures were handled under gold lights to prevent photolysis of bromodeoxyuridine (BrdU)-substituted DNA. Each

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test consisted of concurrent solvent and positive controls and of at least three doses of vinyl toluene; the high dose was limited by toxicity or solubility but did not exceed 5 mg/ml.

In the SCE test without S9, CHO cells were incubated for 26 hours with the study chemical in McCoy's 5A medium supplemented with 10% fetal bovine serum, L-glutamine (2 mM), and antibiotics. BrdU was added 2 hours after culture initiation. After 26 hours, the medium containing the study chemical was removed and replaced with fresh medium plus BrdU and colcemid, and incubation was continued 2 more hours. Cells were then harvested by mitotic shake-off, fixed, and stained with Hoechst 33258 and Giemsa. In the SCE test with S9, cells were incubated with the chemical, serum-free medium, and S9 for 2 hours. The medium was then removed and replaced with medium containing BrdU and no study chemical; incubation proceeded for an additional 26 hours, with colcemid present for the final 2 hours. Harvesting and staining were the same as for cells treated without S9.

In the chromosomal aberration test without S9, cells were incubated in McCoy's 5A medium with the study chemical for 8 hours; colcemid was added, and incubation was continued for 2 hours. The cells were then harvested by mitotic shake-off, fixed, and stained with Giemsa. For the chromosomal aberration test with S9, cells were treated with the study chemical and S9 for 2 hours, after which the treatment medium was removed and the cells were incubated for 10 hours in fresh medium, with colcemid present for the final 2 hours. Cells were harvested in the same manner as for the treatment without S9.

For the SCE test, if significant chemical-induced cell cycle delay was seen, incubation time was lengthened to ensure a sufficient number of scorable cells. The harvest time for the chromosomal aberration test was based on the cell cycle information obtained in the SCE test; if cell cycle delay was anticipated, the incubation period was extended approximately 5 hours.

Cells were selected for scoring on the basis of good morphology and completeness of karyotype (21 ± 2 chromosomes). All slides

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were scored blind, and those from a single test were read by the same person. For the SCE test, 50 second-division metaphase cells were usually scored for frequency of SCEs per cell from each dose; 100 first-division metaphase cells were scored at each dose for the chromosomal aberration test. Classes of aberrations included simple (breaks and terminal deletions), complex (rearrangements and translocations), and other (pulverized cells, despiralized chromosomes, and cells containing 10 or more aberrations).

Statistical analyses were conducted on both the slopes of the dose-response curves and the individual dose points. An SCE frequency 20% above he concurrent solvent control was chosen as a statistically conservative positive response. The probability of this level of difference occurring by chance at one point is less than 0.01; the probability for such a chance occurrence at two dose points is less than 0.001. Chromosomal aberration data are presented as percentage of cells with aberrations. As with SCEs, both the dose-response curve and individual dose points were statistically analyzed. A statistically significant ($p<0.003$) trend test or a significantly increased dose point ($p<0.05$) was sufficient to indicate a chemical effect.

Results Negative
Remarks: In cytogenetic tests with CHO cells, vinyl toluene did not induce SCEs or chromosomal aberrations in either the presence or the absence of Aroclor 1254-induced male Sprague Dawley rat liver S9 (Tables H3 and H4).

Conclusions: Vinyl toluene did not induce sister chromatid exchanges or chromosomal aberrations in CHO cells with or without S9.

Data Quality
Reliability: Acceptable
Remarks: The key parameters (i.e., concentrations, use of positive controls) were appropriate and adequately described.

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CAS # 25013-15-4

HUMAN HEALTH EFFECTS ELEMENTS
Toxicity in Vitro (Gene Mutations) – Study 3

Reference: Toxicology and Carcinogenesis Studies of Vinyl Toluene (Mixed Isomers; 65-71% meta-isomer and 32-35% para-isomer) in F344/N Rats and B6C3F_1 Mice (Inhalation Studies). National Toxicology Program Technical Report Series No. 375, NIH Publication No. 90-2830.

Galloway, S.M., Armstrong, M.J., Reuben, C., Colman, S., Brown, B., Cannon, C., Bloom, A.D., Nakamura, F., Ahmed, M., Duk, S., Rimpo, J., Margolin, B.H., Resnick, M.A., Anderson, B. and Zeiger, E. (1987) Chromosome aberrations and sister chromatid exchanges in Chinese hamster ovary cells. Evaluations of 108 chemicals. Environ. Molec. Mutagen. 10(Suppl. 10):1-175.

TABLE H3. INDUCTION OF SISTER CHROMATID EXCHANGES IN CHINESE HAMSTER OVARY CELLS BY VINYL TOLUENE (a)

Compound	Dose (µg/ml)	Total Cells	No. of Chromo-somes	No. of SCEs	SCEs/ Chromo-some	SCEs/ Cell	Hours in BrdU	Relative SCEs/Cell (percent) (b)
−S9 (c)								
Trial 1--Summary: Negative								
Dimethyl sulfoxide		50	1,046	461	0.44	9.2	27.0	
Vinyl toluene	1.6	50	1,043	442	0.42	8.8	27.0	95.7
	5	50	1,046	431	0.41	8.6	27.0	93.5
	16	50	1,049	457	0.44	9.1	27.0	98.9
	50	50	1,048	431	0.41	8.6	27.0	93.5
Mitomycin C	0.001	50	1,047	719	0.69	14.4	27.0	156.5
Trial 2--Summary: Negative								
Dimethyl sulfoxide		50	1,045	459	0.44	9.2	26.0	
Vinyl toluene	5	50	1,049	431	0.41	8.6	26.0	93.5
	10	50	1,042	454	0.44	9.1	26.0	98.9
	25	6	125	54	0.43	9.0	26.0	97.8
	50	50	1,049	441	0.42	8.8	26.0	95.7
	75	50	1,043	467	0.45	9.3	26.0	101.1
	100	0	--	--		--	--	--
	150	2	42	30	0.71	15.0	26.0	163.0
Mitomycin C	0.0008	10	205	118	0.58	11.8	26.0	128.3
	0.005	10	209	318	1.52	31.8	26.0	345.7
Trial 3--Summary: Negative								
Dimethyl sulfoxide		50	1,046	480	0.46	9.6	26.0	
Vinyl toluene	25	50	1,046	516	0.49	10.3	26.0	107.3
	50	50	1,046	459	0.44	9.2	26.0	95.8
	75	50	1,041	485	0.47	9.7	(d) 35.5	101.0
	100	50	1,041	554	0.53	11.1	(d) 35.5	115.6
Mitomycin C	0.0008	50	1,047	641	0.61	12.8	26.0	133.3
	0.005	10	209	310	1.48	31.0	26.0	322.9
+S9 (e)								
Trial 1--Summary: Negative								
Dimethyl sulfoxide		50	1,046	392	0.37	7.8	26.0	
Vinyl toluene	5	50	1,049	388	0.37	7.8	26.0	100.0
	16	50	1,041	420	0.40	8.4	26.0	107.7
	50	50	1,043	367	0.35	7.3	26.0	93.6
Cyclophosphamide	0.3	50	1,047	708	0.68	14.2	26.0	182.1
	0.6	10	210	208	0.99	20.8	26.0	266.72

TABLE H3. INDUCTION OF SISTER CHROMATID EXCHANGES IN CHINESE HAMSTER OVARY CELLS BY VINYL TOLUENE (Continued)

Compound	Dose (µg/ml)	Total Cells	No. of Chromosomes	No. of SCEs	SCEs/ Chromosome	SCEs/ Cell	Hours in BrdU	Relative SCEs/Cell (percent) (b)
+S9 (e)								
Trial 2--Summary: Negative								
Dimethyl sulfoxide		50	1,039	403	0.39	8.1	26.0	
Vinyl toluene	10	50	1,041	402	0.39	8.0	26.0	98.8
	25	50	1,045	360	0.34	7.2	26.0	88.9
	50	50	1,034	440	0.43	8.8	26.0	108.6
	75	50	1,040	410	0.39	8.2	26.0	101.2
Cyclophosphamide	0.3	50	1,046	562	0.54	11.2	26.0	138.3
	0.6	10	210	142	0.68	14.2	26.0	175.3

(a) Study performed at Environmental Health Research and Testing, Inc. SCE = sister chromatid exchange; BrdU = bromodeoxyuridine. A detailed description of the SCE protocol is presented by Galloway et al. (1987). Briefly, Chinese hamster ovary (CHO) cells were incubated with study compound or solvent as described in (c) and (e) below, and cultured for sufficient time to reach second metaphase division. Cells were then collected by mitotic shake-off, fixed, air dried, and stained.
(b) SCEs/cell of culture exposed to study chemical relative to those of culture exposed to solvent.
(c) In the absence of S9, CHO cells were incubated with study compound or solvent for 2 hours at 37° C. Then BrdU was added, and incubation was continued for 24 hours. Cells were washed, fresh medium containing BrdU and colcemid was added, and incubation was continued for 2-3 hours.
(d) Because some chemicals induce a delay in the cell division cycle, harvest times are occasionally extended to maximize the proportion of second division cells available for analysis.
(e) In the presence of S9, cells were incubated with study compound or solvent for 2 hours at 37° C. Cells were then washed, and medium containing BrdU was added. Cells were incubated for a further 26 hours, with colcemid present for the final 2-3 hours. S9 was from the liver of Aroclor 1254-induced male Sprague Dawley rats.

TABLE H4. INDUCTION OF CHROMOSOMAL ABERRATIONS IN CHINESE HAMSTER OVARY CELLS BY VINYL TOLUENE (a)

−S9 (b)					+S9 (c)				
Dose (µg/ml)	Total Cells	No. of Abs	Abs/ Cell	Percent Cells with Abs	Dose (µg/ml)	Total Cells	No. of Abs	Abs/ Cell	Percent Cells with Abs
Harvest time: 12.0 h					Harvest time: 13.3 h				
Dimethyl sulfoxide					Dimethyl sulfoxide				
	100	0	0.0	0.0		100	1	0.01	1.0
Vinyl toluene					Vinyl toluene				
1.6	100	1	0.01	1.0	5	100	1	0.01	1.0
5	100	2	0.02	2.0	16	100	1	0.01	1.0
16	100	0	0.00	0.0	50	100	0	0.00	0.0
50	100	2	0.02	2.0					
Summary: Negative					Summary: Negative				
Mitomycin C					Cyclophosphamide				
0.125	100	5	0.05	5.0	15	100	10	0.10	10.0
0.25	100	27	0.27	19.0	50	50	28	0.56	48.0

(a) Study performed at Environmental Health Research and Testing, Inc. Abs = aberrations. A detailed presentation of the technique for detecting chromosomal aberrations is found in Galloway et al. (1987). Briefly, Chinese hamster ovary cells were incubated with study compound or solvent as indicated in (b) and (c). Cells were arrested in first metaphase by addition of colcemid and harvested by mitotic shake-off, fixed, and stained in 6% Giemsa.
(b) In the absence of S9, cells were incubated with study compound or solvent for 8-10 hours at 37° C. Cells were then washed, and fresh medium containing colcemid was added for an additional 2-3 hours followed by harvest.
(c) In the presence of S9, cells were incubated with study compound or solvent for 2 hours at 37° C. Cells were then washed, medium was added, and incubation was continued for 8-10 hours. Colcemid was added for the last 2-3 hours of incubation before harvest. S9 was from the liver of Aroclor 1254-induced male Sprague Dawley rats.

SURROGATE STUDIES USING P-METHYLSTYRENE

ROBUST SUMMARY
STYRENE, AR-METHYL-(VINYL TOLUENE)
CAS # 25013-15-4

HUMAN HEALTH EFFECTS ELEMENTS
Toxicity to Reproduction – Study 1

Test Substance

Remarks:	p-methylstyrene Purity: 100% assumed for dosage calculations Solvent carrier: Olive oil (1.0 ml/kg) Contaminants: None reported Chemical formula: C_9H_{10}

Method

Method/Guideline:	Not referenced
Test Type:	Two generation study
GLP:	Yes
Year of study:	1980
Species:	Rat
Strain:	CD-1
Sex:	Both
Number per dose:	25
Route of Administration:	Oral
Concentrations:	25, 200, 500, and 600 mg/kg/day
Duration:	Once daily, seven days/week, for 404 days
Control Group:	Yes
Premating exposure period for females:	P, 14 weeks; F_1, 17 weeks
Premating exposure period for males:	P, 14 weeks; F_1, 17 weeks
Statistical Method:	ANOVA, Kruskal-Wallis test
Remarks:	P-methylstyrene (PMS) was administered by daily oral gavage in olive oil (volume of 1.0 ml/kg) to male and female Sprague-Dawley CD rats for 2 generations at daily doses of 25, 200, and 500 mg/kg/day. PMS was administered to another group of rats at 600 mg/kg/day for 1 generation (500 mg/kg/day plus controls were added when excess mortality was seen in 600 mg/kg/day). Another group of rats which received olive oil without PMS for 2 generations served as controls. In the P generation (F_o), rats were 5 weeks old at the initiation of dosing (males 45 to 65 grams, females 40 to 60 grams). The number of males and females in controls and 500 mg/kg/day were 30 each, and the number of

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males and females in 25, 200, and 600 mg/kg/day were 25. After 14 weeks of dosing of P rats, males and females were placed in cohabitation, one male per each female. The rats continued to receive PMS during cohabitation, gestation, delivery, and lactation. At weaning, 5 male and 5 female pups from each treatment group were selected for gross necropsy; after fixation and processing, tissues from the pups from the control and 500 mg/kg/day were examined microscopically. Forty male and forty female F_1 weanling pups from each treatment group were selected for continued treatment with PMS (20 males and 20 females were obtained for controls). Treatment groups were administered PMS for 17 weeks (25, 200, and 500 mg/kg/day) before cohabitation, during cohabitation, gestation, delivery, and lactation. At weaning, 5 male and 5 female F_2 pups from each litter were selected for gross necropsy; after fixation and processing, tissues from the pups from the control and 500 mg/kg/day were examined microscopically. F_1 and F_2 generation litters were examined as soon as possible after natural delivery. Pup viability and body weights of live pups were recorded at birth (day 1) and when pups were 4, 7, 14, and 21 days old. Litters were evaluated for maternal and pup behavior during the lactation period, when pup body weights were recorded. Ten adult males and 25 adult females killed by design from each dose were also necropsied, plus animals found dead, and tissues from the control and 500 mg/kg/day were examined microscopically. The rats in all treatment groups were observed daily for mortality and clinical signs of toxicity; body weight and food consumption were measured weekly.

Results

Parental NOAEL:	200 mg/kg/day
Parental NOAEL effect:	Mortality
Parental LOAEL:	500 mg/kg/day
Parental LOAEL effect:	Mortality
F_1 NOAEL:	200 mg/kg/day

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F_1 NOAEL effect:	Mortality
F_1 LOAEL:	500 mg/kg/day
F_1 LOAEL effect:	Mortality
F_2 NOAEL:	$\geq$500 mg/kg/day
F_2 NOAEL effect:	Mortality or reproductive parameters
F_2 LOAEL:	>500 mg/kg/day
F_2 LOAEL effect:	Mortality or reproductive parameters
Actual dose received by dose level by sex:	25.5, 196.0, 508.0, and 887.2 mg/ml
Parental/F_1 Data:	Deaths were 1/80, 2/80, 4/80, and 27/80 for control, 25, 200, and 500 mg/kg/day
Offspring Data:	Administration of 500 mg/kg/day to F_1 generation male and female rats resulted in a small increase (not statistically significant) in the incidence of stillborn pups and in a slight increase in pup mortality.
Statistical results:	All statistically significant results (below) were at $p<0.05$.
Remarks:	Mortality in the F_o generation for male rats was as follows: 2/54, 0/25, 2/25, 10/30, and 12/25 for control, 25, 200, 500, and 600 mg/kg/day, respectively. Mortality in the F_o generation for female rats was as follows: 2/55, 1/25, 3/27, 7/30, and 14/25 for control, 25, 200, 500, and 600 mg/kg/day, respectively. Physical signs observed in F_o generation rats in the 25, 200, 500, and 600 mg/kg/day dosage groups and attributed to treatment with PMS generally occurred in a dosage-related pattern and included excess salivation, and hyperactivity/vocalization. Excess urination during handling, "tip-toe" walk, chromorrhinorrhea/nasal discharge and pilo-erection also occurred in a dosage-related pattern in male rats in these four PMS-treated groups, and in female rats in the 200, 500, and 600 mg/kg/day dosage groups. Rales/dyspnea and urine-stained abdominal fur were considered dose-related in 500 and 600 mg/kg/day dosage group rats. During the 14 week precohabitation period, dose-related inhibition of average body weight gain was observed in F_o generation male and female rats administered 200, 500, and 600 mg/kg/day, compared with controls. The effect was slightly more severe in male than in female rats and gradually disappeared in rats in the 200 and 500 mg/kg/day groups. Average weekly body weights in

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HUMAN HEALTH EFFECTS ELEMENTS
Toxicity to Reproduction – Study 1

rats administered 25 mg/kg/day were comparable to controls during the first 14 weeks.

During the postcohabitation period in F_o generation male rats, inhibition of body weight gain was observed in 500 and 600 mg/kg/day groups, compared to controls. Dose-related inhibition of average body weight gain during gestation and lactation occurred in F_o generation female rats administered 600 mg/kg/day, compared to controls.

During the precohabitation period, dose-related inhibition of average weekly food consumption occurred in F_o generation male rats administered 500 and 600 mg/kg/day, compared with controls. Later in the study, male rats administered 200 mg/kg/day, and female rats administered 600 mg/kg/day had significantly higher average weekly food consumption values than controls; the biological importance of this observation is not presently known. Average weekly food consumption of rats in the 25 mg/kg/day group was comparable to vehicle controls.

During the firs week of the postcohabitation period, F_o generation male rats in 200, 500, and 600 mg/kg/day consumed more food controls. This effect disappeared after week 1 and may have been associated with the return of the rats from cohabitation to individual housing.

During gestation in F_o generation female rats, decreased average food consumption was observed in 500 and 600 mg/kg/day, compared with controls. The effect gradually disappeared. During the lactation period, a minimal decrease in average food consumption was observed in 500 and 600 mg/kg/day rats, compared with controls.

Compared with controls, treatment of F_o generation male and female rats with 600 mg/kg/day resulted in a decrease in the average number of liveborn pups/litter, and with 500 and 600 mg/kg/day in a small increase in pup mortality and decrease in

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average pup body weight per litter. Postweaning and during the period of PMS-administered evaluation, PMS was considered the cause of death of male and female rats in the 200 and 500 mg/kg/day groups.

A summary of mating and fertility data for the F_o generation is as follows (presented for control I, control II, 25, 200, 500, ad 600 mg/kg/day, respectively): Fertility Index (%, pregnant/total); 63.6, 93.1, 88.0, 91.7, 90.9, and 86.7. Duration of gestation (mean days), 23.5, 23.0, 23.6, 23.2, 23.2, and 23.2. Gestation index (live litters day 1, %), 85.7, 96.3, 95.4, 100, 89.5, and 91.7. Implantations (mean), 11.4, 12.9, 11.6, 12.0, 12.2, and 11.0.

Mortality in the male F_1 generation was as follows: 0/40, 0/40, 1/40, and 19/40 for controls, 25, 200, and 500 mg/kg/day, respectively. Mortality in the female F_1 generation was as follows: 1/40, 2/40, 3/40, and 8/40 for controls, 25, 200, and 500 mg/kg/day, respectively. Physical signs observed in F_1 generation rats were similar to those observed in the F_o generation rats. Dose-related signs observed generally occurred in a dose-related pattern. Signs observed in male and female rats which were considered related to administration of 25, 200, and 500 mg/kg/day, compared with controls, included excess salivation, hyperactivity/vocalization, excess urination during handling, “tip-toe” walk, chromorrhinorrhea/nasal discharge and hypersensitivity to touch. Male rats in all dose groups had pilo-erection and ungroomed coat; in female rats this sign was associated only with the 500 mg/kg/day group. Thin appearance in male rats administered 200 and 500 mg/kg/day, and in female rats administered 25, 200, and 500 mg/kg/day was attributed to the test substance. Urine-stained abdominal fur in 500 mg/kg/day group male rats, and in 200 and 500 mg/kg/day group female rats was also considered dose-related. Decreased motor activity and rales/dyspnea in both male and female rats were considered effects of the 500 mg/kg/day group. During the 17-week precohabitation period, dose-related inhibition of average body weight gain was

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observed in F_1 generation male rats in 25, 200, and 500 mg/kg/day, and in F_1 generation female rats in 500 mg/kg/day, as compared to controls. F_1 generation female rats in 25 mg/kg/day had slightly smaller average body weight gains than controls. This observation was not attributed to the test substance as a dose-response was not observed. Administration of 200 mg/kg/day, compared to controls, frequently resulted in greater average weekly body weight gains in F_1 generation female rats. This biological significance of this observation is currently unknown.

Dose-related inhibition of average body weight gain persisted during the postcohabitation period for F_1 generation male rats in the 25, 200, and 500 mg/kg/day groups, compared with controls.

For F_1 generation female rats, dose-related lower average body weights persisted for the 500 mg/kg/day group, compared with controls, during the gestation, lactation, and postweaning periods.

During the precohabitation period, weeks 1 through 17 of the study, and the postcohabitation period, weeks 21 through 26 of the study, dose-related decrease in average weekly food consumption occurred in 500 mg/kg/day group F_1 generation male rats, compared with controls. In F_1 generation female rats in 25 and 200 mg/kg/day, average weekly food consumption was increased, as compared with controls, during the precohabitation and gestation period. The observations disappeared during the lactation period. Average weekly food consumption of 500 mg/kg/day group female rats was similar to controls during the precohabitation, gestation, and lactation periods.

Administration of 500 mg/kg/day to F_1 generation male and female rats, compared to controls, resulted in a small increase in the incidence of stillborn pups and in a slight increase in pup mortality. Differences were not significant at $p<0.05$.

Treatment of F_1 generation rats with 25, 200, and 500 mg/kg/day, compared with controls, did not result in alteration of the

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percentage of rats which mated, the number of days required to mate, the duration of gestation, the fertility index, the gestation index, pup mortality per litter, pup sex ratio or pup average body weight per litter.

A summary of mating and fertility data for F_1 generation is as follows (presented as control I, control II, 25, 200, and 500 mg/kg/day, respectively); Fertility Index (pregnant/total %), 88.9, 84.2, 91.4, 86.5, and 83.9. Duration of gestation (mean days), 22.2, 22.1, 22.2, 22.4, and 22.4. Gestation index (live litters day 1, %), 100, 100, 100, 100, and 92.3. Implantations (mean), 11.0, 12.2, 10.9, 10.4, and 10.5.

A summary of the pathology report is as follows: Dosing observations, gross necropsy findings, and extensive microscopic examination all support the conclusion that many deaths were caused by aspiration or intubation accidents. From the microscopic evaluation it is certain that nearly all animals in the F_1 high-level group (possibly all PMS groups) were unintentionally subjected to periodic (perhaps frequent) small aspirations of PMS/oil, with rapid or overnight death following the aspiration or intubation of a larger amount.

Essentially all the tracheobronchial lesions are regarded as part of one spectrum of changes induced by unintended periodic local exposure of the tracheobronchial mucosa to small amounts of PMS/olive oil, the exposure occurring via repetitive tiny aspirations or intubation of test material. Once developed, some primary lesions caused secondary changes: 1) mucus retention was accentuated by the simultaneous loss of cilia in the upper bronchi and trachea, and hypertrophy/hyperplasia of mucous cells in the lower bronchi; 2) segmental occlusion/obliteration of bronchioles was associated with an increased incidence of areas of atelectasis; and 3) all primary changes may have contributed to the

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development of a fatal severity of acute bronchitis and bronchopneumonia in seven of the animals found dead.

Except for the local effects on trachea and lungs of unintended repetitive aspiration/intubation of test article, there was no morphologic evidence of any effect of PMS/olive oil in F_o and F_1 adult rats dosed daily by gavage at a level of 500 mg/kg/day. In F_1 and F_2 weanling pups, there was no morphological evidence of any effect of PMS. Histopathologic examinations revealed no neoplastic or preneoplastic changes.

Specifically, morphologic examinations of F_o adults (gross only) and F_1 adults and F_1 and F_2 pups (gross and microscopic) revealed no evidence of any treatment effect on the organs of reproduction.

Conclusions: When PMS was administered to rats at a dose of 600 mg/kg/day, there were adverse effects on both the parents (increased mortality, reduced weight gain, excitability) and on the pups (slightly smaller litter size, slightly more mortality during the first 4 days after birth, slightly less weight gain). When PMS was administered at 500 mg/kg/day, similar adverse effects (mortality, reduced weight gain, excitability) were seen in the parents, but less pronounced effects on pups (slightly smaller litter size and slightly more mortality during the first 4 days after birth in the first generation only). When PMS was administered at 200 mg/kg/day, there were no effects on the success of reproduction or health of offspring, and only a slight effect on the behavior of the parents (excitability).

There was no effect of treatment with PMS on the viability of pups from dams dosed at 25 or at 200 mg/kg/day. In addition, there was no effect on the mating, fertility, gestation, delivery of pups, or lactation index (survival to weaning of pups alive at day 4). The growth rate of pups from dams dosed at 25, 200, or 500 mg/kg/day was not different from the pups from the control dams. Pups from dams dosed at 600 mg/kg/day showed a 10% reduction in body weight, both at birth and at weaning, as compared to the controls.

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The effects observed in the offspring of the rats dosed at 500 or 600 mg/kg/day probably resulted from maternal toxicity, not from effects of PMS directly on the reproductive process. It is concluded that at doses which are not maternally toxic, PMS does not interfere with the reproductive process in rats.

Data Quality:

Reliability: Acceptable

Remarks: The key parameters (doses, number of animals, observations, etc.) were appropriate and described in the study.

Reference: Reproductive Effects of p-Methylstyrene Administered Orally Via Gavage to Crl:COBSCD (SD)BR Rats for Two Generations, Mobil Environmental and Health Science Laboratory Study No: 2160-80, July 16, 1984. M2161-80.

General: This study is acceptable as a surrogate study for vinyl toluene (mixed isomers, 65%-71% meta and 32%-35% para).

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Test Substance

Remarks:	p-methylstyrene Purity: 100% assumed for dosage calculations Solvent carrier: Olive oil (1.0 ml/kg) Contaminants: None reported Chemical formula: C_9H_{10}

Method

Method/Guideline:	Not referenced
GLP:	Unknown
Year of study:	1978
Species:	Rat
Strain:	Sprague-Dawley
Sex:	Female
Number per dose:	20
Route of Administration:	Oral
Days of Gestation:	6-15
Concentrations:	60, 190, and 600 mg/kg/day
Duration:	Once daily, seven days/week
Control Group:	Yes
Statistical Method:	Incidences of occurrence (expressed as %) were analyzed using 95% confidence intervals for proportions or by computation of exact probabilities. Body weights analyzed by ANOVA and least significance difference test was used for comparisons.
Remarks:	Sexually mature female Sprague-Dawley rats (220 to 230 g body weight) were mated in 1:1 with males in sufficient numbers to assign a minimum of 20 pregnant animals per group. Beginning on day 6 of gestation and continuing daily through day 15 of gestation, the test substance was suspended in corn oil and administered by oral intubation (volume, 1.0 ml/kg) to the pregnant females at concentrations of 60, 190, and 600 mg/kg/day. A negative control (corn oil, 1 ml/kg/day) and positive control (aspirin, 250 mg/kg/day) were also employed. On day 20 of gestation, all females were killed by a 5-10 minute exposure to chloroform vapors. The uterine contents of each were removed

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and the reproductive performance recorded. The urogenital tract of each female was examined for normal morphology. Body weights of all females were recorded on days 0, 6, 11, 15, and 20 of gestation. All animals were observed daily for signs of toxicity and a record maintained. At the time of sacrifice on day 20, the following observations were recorded for each female: numbers of corpora lutea, implantation sites, resorption sites, live and dead fetuses, sex or fetuses, and body weights of fetuses.

At the time of uterine examination, all fetuses were examined grossly for the presence of external congenital abnormalities. One-third of the fetuses from each litter were randomly selected and placed in Bouin's solution for detailed visceral examination employing Wilson freehand slicing technique. Any fetus showing external abnormalities was selected for examination by this technique. The remaining fetuses were eviscerated, fixed in 70% isopropyl alcohol, macerated in a 2% potassium hydroxide solution, stained with Alizarin-Red S dye, cleared in glycerin, and examined under low power magnification for skeletal anomalies and ossification variations. Each fetus was processed, examined and stored for possible further examination in a manner retaining the identity of both dam number and uterine position.

Results:

Maternal NOAEL:	≥600 mg/kg/day
Maternal NOAEL effect:	Mortality, body weight, pregnancy
Maternal LOAEL:	>600 mg/kg/day
Maternal LOAEL effect:	Mortality, body weight, pregnancy
Developmental NOAEL:	≥600 mg/kg/day
Developmental NOAEL effect:	Skeletal or soft tissue abnormalities
Developmental LOAEL:	>600 mg/kg/day

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Developmental LOAEL effect:	Skeletal or soft tissue abnormalities
Actual dose:	Not available
Maternal data with dose level:	There were no differences in body weights, pregnancy, implantation, numbers of live or dead fetuses, or numbers of resorptions per dam at any test level.
Fetal data with dose level:	An increase in litters with rudimentary ribs at 60 mg/kg/day and extra ribs at 190 mg/kg/day were observed. In addition, fetuses exhibited an increase in incomplete ossification of vertebrae.
Statistical results:	Statistical significance (see below) was observed at $p<0.05$ in several parameters in the positive controls and a few in the 60 and 190 mg/kg/day groups.

Results:

Remarks: There were no significant differences in pregnancy, implantation, numbers of live fetuses, numbers of dead fetuses, or numbers of resorptions per dam between any test level and negative control. The test substance showed no dose-related effects on reproductive performance of fetal weight. Females exhibited body weights and weight gains no different from the negative control animals throughout gestation.

The abnormalities noted in fetuses whose dams received 60, 190, or 600 mg/kg/day were generally variations rather than malformations. The data showed an increase in litters with rudimentary ribs at 60 mg/kg/day and an increase in extra ribs at 190 mg/kg/day. In addition, fetuses exhibited an increase in the incomplete ossification of vertebrae at 60 and 190 mg/kg/day. However, fetuses from dams treated at 600 mg/kg/day showed skeletal effects no different from the vehicle control. No dose-related skeletal effects were attributed to treatment with the test substance. Soft tissue examinations revealed no significant differences in type or frequency of anomalies between the control and any test group.

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Conclusions: When orally administered to pregnant Sprague-Dawley rats from day 6 through day 15 of gestation at levels of 0, 60, 190, and 600 mg/kg/day, PMS had no dose-related effects on reproduction, gestation, or on skeletal or soft tissue anomalies of fetuses.

Data Quality:
Reliability: Acceptable
Remarks: The key parameters (i.e., number of doses, animals, observations, etc.) were appropriate and described in the study.

Reference: Teratologic Evaluation of MCTR-92-78 in Sprague-Dawley Rats, Food and Drug Research Laboratories, Inc. No. 5924, January 19, 1979.

General: This study is acceptable as a surrogate study for vinyl toluene (mixed isomers, 65%-71% meta and 32%-35% para).

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Developmental Toxicity/Teratogenicity – Study 2

Test Substance

Remarks:	p-methylstyrene Purity: 100% assumed for dosage calculations Solvent carrier: Olive oil (5.0 ml/kg) Contaminants: None reported Chemical formula: C_9H_{10}

Method

Method/Guideline:	Not referenced
GLP:	Yes
Year of study:	1980
Species:	Rat
Strain:	CD-1
Sex:	Female
Number per dose:	25
Route of Administration:	Oral
Days of Gestation:	6-19
Concentrations:	50, 300, and 600 mg/kg/day
Duration:	Once daily, seven days/week
Control Group:	Yes
Statistical Method:	Chi-square and/or Fisher's exact for the number of litters with malformations; Mann-Whitney U-test for early/late resorptions; ANOVA with Dunnett's for viable fetuses, implantations, corpora lutea, body weights
Remarks:	Sexually mature, virgin female Charles River COBS CD rats (approximately 12 weeks old) were mated in 1:1 with males in sufficient numbers to assign a minimum of 25 pregnant animals per group. Beginning on day 6 of gestation and continuing daily through day 19 of gestation, the test substance was suspended in corn oil and administered by oral gavage (volume, 5.0 ml/kg) to pregnant females at a single daily dose of 50, 300, and 600 mg/kg/day. A control group received the vehicle only on a comparable regimen at a volume of 5 ml/kg. Prior to treatment, the dams were observed daily for mortality and overt changes in appearance and behavior. They were observed for mortality and

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clinical signs of toxicity on days 6 through 20 of gestation. Individual maternal body weights were recorded on gestation days 0, 6, 9, 12, 16, and 20. On gestation day 20, all females were sacrificed by carbon dioxide inhalation. Immediately following sacrifice, the abdominal cavity was opened to expose the uterus and ovaries. The uterus was excised and weighed prior to removal of the fetuses. The number and location of viable and nonviable fetuses, early and late resorptions, and the number of total implantations and corpora lutea were recorded. The abdominal and thoracic cavities and organs of the females were grossly examined for pathological changes and the carcasses discarded. Uteri from females that appeared nongravid were opened and placed in 10% ammonium sulfide for confirmation of pregnancy status.

All fetuses were individually weighed and examined for external malformations and variations, including the palate and eyes. Each fetus was externally sexed and individually numbered and tagged for identification. Approximately one-half of the fetuses were placed in Bouin's fixative for subsequent visceral examination by razor-blade sectioning. The remaining one-half of the fetuses were fixed in alcohol, macerated in potassium hydroxide and stained with Alizarin Red S for subsequent skeletal examination.

Results:

Maternal NOAEL:	≥600 mg/kg/day
Maternal NOAEL effect:	Mortality, pregnancy
Maternal LOAEL:	>600 mg/kg/day
Maternal LOAEL effect:	Mortality, pregnancy
Developmental NOAEL:	≥600 mg/kg/day
Developmental NOAEL effect:	Skeletal or soft tissue abnormalities
Developmental LOAEL:	>600 mg/kg/day

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Developmental LOAEL effect:	Skeletal or soft tissue abnormalities
Actual dose:	Not available
Maternal data with dose level:	A dose-related reduction in mean maternal body weight gain was observed in all treated groups when compared to the control group. Apparently, these decreases were not statistically significant.
Fetal data with dose level:	A statistically significant reduction in mean fetal body weight was observed in all treated groups compared to controls. Mean number of corpora lutea was significant at 600 mg/kg/day.
Statistical results:	Mean fetal body weight for all treatment groups significant at $p<0.05$ or $p<0.01$ (600 mg/kg/day), mean number of corpora lutea for 600 mg/kg/day significant at $p<0.05$.

Results:

Remarks: No biologically meaningful differences in appearance or behavior were noted in any of the treatment groups. At necropsy all animals were found to be internally normal with the exception of two control animals (yellow fluid in intestines, 2 mm hydrocele on the left oviduct) and one animal in 600 mg/kg/day (hydronephrosis). A dose-related reduction in mean body weight gain was noted over the entire treatment period for all treated groups compared to control. A similar pattern was also observed for the adjusted body weight gain (female weight exclusive of uterus and contents). Statistical significance was not reported.

A statistically significant reduction in mean fetal body weight was observed in all treated groups when compared to controls. However, the reductions may not be indicative of a true compound effect as the reported values for all treated groups exceeded the historical control value, and the test control was unusually high. There was a statistically significant reduction in the mean number of corpora lutea in the 600 mg/kg/day when compared to controls. However, this was not considered treatment-related because

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ovulation and implantation occur prior to test article administration.

There were no biologically meaningful or statistically significant differences in the mean number of viable fetuses, early or late resorptions, postimplantation loss, total implantations or the fetal sex distribution in any of the treated groups when compared to the control group. The mean number of corpora lutea in the 50 and 300 mg/kg/day groups was also comparable o the control value. Nonviable fetuses were not observed in any of the study groups including the controls.

No malformations were observed in the control, 50 or 300 mg/kg/day groups. One malformation, meningocele, was observed in one fetus from one litter in the 600 mg/kg/day. The number of fetuses and litters with genetic or developmental variations in all treated groups was comparable to the control group.

Conclusions: A dose-related reduction in mean maternal body weight gain was noted over the entire treatment period for all treated groups when compared with the control group. A similar pattern was observed for the adjusted body weight gain (female weight exclusive of uterus and contents). The most notable reduction in mean maternal body weight gain occurred in the 300 and 600 mg/kg/day groups during the first three days of treatment period (gestation days 6 through 9) and the last four days of the study period (gestation days 16 through 20). However, these results are apparently not statistically significant since the report does not indicate statistical significance. A reduction of mean fetal body weight occurred in all treated groups when compared with the control group. However, the importance of this reduction is unclear since the mean fetal body weights of all groups including the control were above the average as reported by the mean historical control of this laboratory. The exceptionally high control value (outside the range of historical control) may have contributed to the statistical significance between treated and control groups.

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Treatment with p-methylstyrene did not produce a teratogenic response when administered orally to pregnant rats at a dosage level of 600 mg/kg/day or less.

Data Quality:
Reliability: Acceptable
Remarks: The key parameters (i.e., number of doses, animals, observations, etc.) were appropriate and described in the study.

Reference: Teratology Study in Rats (MCTR-302-79), International Research and Development Corporation Study No.: 450-025, October 8, 1981. M3020-79.
Pilot Teratology Study in Rats (MCTR-309-79), International Research and Development Corporation Study No.: 450-024, January 14, 1981. M3090-79.

General: Pregnant Charles River COBS CD rats were used to establish dosage levels of Sample 01188001 (p-methylstyrene) for a teratology study. Dosage levels of 0, 50, 200, 400, 800, and 1200 mg/kg/day were administered orally by gavage as a single daily dose on days 6 through 19 of gestation, at a constant vehicle volume of 5 ml/kg. The control group received the vehicle only, olive oil, on a comparable regimen. Uterine examinations were performed on all surviving females on gestation day 20. Survival in the control, 50, 200, 400, and 800 mg/kg/day dosage group was 100%. One female in the 1200 mg/kg/day treatment group died on gestation day 10. A cause of death could not be determined at necropsy. There were no biologically meaningful differences in appearance and behavior attributable to treatment with Sample 01188001 in the 50, 200, 400, or 800 mg/kg/day treatment groups when compared to the control group. Increases in yellow staining around the mouth and anogenital region and excessive salivation were observed in a few rats in the 1200 mg/kg/day dosage group. A slight decrease in mean maternal body weight gain in the 50 and 200 mg/kg/day treatment groups, a moderate decrease in the 400 and 800 mg/kg/day dosage groups and a severe reduction in the

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1200 mg/kg/day group were noted. These effects were dose related. When compared to the control group, there were no biologically meaningful differences in mean uterine examination values in any of the Sample 01188001 treatment groups.
Based on these results, a dosage level of 800 mg/kg/day would be considered excessive for a teratology study with rats with p-methylstyrene.

This pilot study was acceptable for determining doses for a teratology study.

In addition, the main study is acceptable as a surrogate study for vinyl toluene (mixed isomers, 65%-71% meta and 32%-35% para).

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Developmental Toxicity/Teratogenicity – Study 3

Test Substance

Remarks:	p-methylstyrene Purity: 97% Solvent carrier: None Contaminants: None reported Chemical formula: C_9H_{10}

Method

Method/Guideline:	Not referenced
GLP:	Yes
Year of study:	1980
Species:	Rabbit
Strain:	Dutch
Sex:	Female
Number per dose:	16
Route of Administration:	Oral
Days of Gestation:	6-27
Concentrations:	50, 100, and 150 mg/kg/day
Duration:	Once daily, seven days/week
Control Group:	Yes
Statistical Method:	Chi-square and/or Fisher's exact test for male to female fetal sex distribution and number of litters with malformations, Mann-Whitney U-test for early and late resorptions and postimplantation loss, ANOVA with Dunnett's test for other endpoints.
Remarks:	Sexually mature, virgin female Dutch Belted rabbits (6 months of age, 2,194 to 2.970 kg) were inseminated and ovulation was induced by injection of chorionic gonadotropin. Prior to insemination, females were randomly assigned to one control group and three treatment groups consisting of 16 rabbits each. The test substance was dispensed daily and administered under a ventilation hood at dosage levels of 50, 100, and 150 mg/kg/day at total dosage volumes of 0.056, 0.112, and 0.169 ml/kg, respectively. The test article administration began on day 6 and continued up to and including day 27 of gestation. The control group received distilled water only on a comparable regimen at a

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total dosage volume equivalent to that of the highest dosage group (0.169 ml/kg).

Prior to treatment, the females were observed daily for mortality and overt changes in appearance and behavior. The females were observed daily for mortality and clinical signs of toxicity on days 6 through 28 of gestation. A gross necropsy was performed on all rabbits not surviving to the scheduled sacrifice in an attempt to determine the cause of death. Tissues were preserved in 10% neutral buffered formalin only as deemed necessary by gross findings. The fetuses from these dams were examined externally and preserved in 10% neutral buffered formalin. Individual maternal body weights were recorded on gestation days 0, 6, 12, 18, 24, and 28.

On gestation day 28, all surviving females were sacrificed by injection of an overdose of sodium pentobarbital. Immediately following sacrifice, the uterus was excised and weighed and the fetuses were removed. The number and location of viable and nonviable fetuses, early and late resorptions, and the number of total implantations and corpora lutea were recorded. The abdominal and thoracic cavities and organs of the dams were examined for grossly evident morphological changes and the carcasses discarded. Uteri from females that appeared nongravid were opened and placed in a 10% ammonium sulfide solution for confirmation of pregnancy status.

All fetuses were individually weighed and examined for external malformations and variations, including the palate and eyes. Each fetus was dissected, internally sexed and examined for visceral malformations and variations, including the brain by a mid-coronal slice. The eviscerated, skinned fetuses were fixed in alcohol, macerated in potassium hydroxide and stained with Alizarin Red S for subsequent skeletal examination. Fetal findings were classified as malformations or genetic or developmental variations.

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Results:

Maternal NOAEL:	≥150 mg/kg/day
Maternal NOAEL effect:	Reproductive parameters
Maternal LOAEL:	>150 mg/kg/day
Maternal LOAEL effect:	Reproductive parameters
Developmental NOAEL:	≥150 mg/kg/day
Developmental NOAEL effect:	Skeletal or soft tissue abnormalities
Developmental LOAEL:	>150 mg/kg/day
Developmental LOAEL effect:	Skeletal or soft tissue abnormalities
Actual dose:	Not available
Maternal data with dose level:	None observed.
Fetal data with dose level:	None observed.
Statistical results:	None reported.
Remarks:	There were no biologically meaningful differences in mean maternal body weight gain in any of the treated groups when compared to controls. A slight reduction in mean maternal body weight gain was noted in the 100 mg/kg/day group. However, this was considered due to random occurrence as no corresponding trend was noted in the 150 mg/kg/day group. There were no biologically meaningful differences in the mean numbers of corpora lutea, total implantations, early or late resorptions, postimplantation loss, viable fetuses, the fetal sex distribution or mean fetal body weight in any treatment groups compared to controls. A slight reduction in the mean number of total implantations with a corresponding decrease in the mean number of viable fetuses was observed at the 150 mg/kg/day level. These reductions were probably the result of a decrease in the mean number of corpora lutea which was also seen at this level. However, this was not considered treatment-related as ovulation

SURROGATE STUDIES USING P-METHYLSTYRENE

ROBUST SUMMARY
STYRENE, AR-METHYL-(VINYL TOLUENE)
CAS # 25013-15-4

HUMAN HEALTH EFFECTS ELEMENTS
Developmental Toxicity/Teratogenicity – Study 3

occurred prior to test article administration. Nonviable fetuses were not observed in any of the study groups.

There were no biologically meaningful or statistically significant differences in the number of litters with malformations in any treatment group compared to controls. No malformations were observed in the 150 mg/kg/day group. In addition, there were no biologically meaningful differences in the number of fetuses (and litters) with genetic or developmental variations in any treatment group compared to controls.

Conclusions: Ten rabbits died prior to scheduled sacrifice between days 9 and 21 of gestation. An intubation error was cited as the probable cause of death of four rabbits; one each in the 50 and 150 mg/kg/day group and two in the 100 mg/kg/day group. Pneumonia was established as the cause of death for five animals; two each in the 50 and 100 mg/kg/day groups and one in the 150 mg/kg/day group. One animal at the 100 mg/kg/day group died without apparent cause.

Treatment with p-methylstyrene did not produce a teratogenic response when administered to pregnant rabbits at a dosage level of 150 mg/kg/day or less.

Data Quality:
Reliability: Acceptable
Remarks: The key parameters (i.e., number of doses, animals, observations, etc.) were appropriate and described in the study.

Reference: Teratology Study in Rabbits (MCTR-303-79), International Research and Development Corporation Study No.: 450-029, January 6, 1982. M3030-79.
Pilot Teratology Study in Rabbits (MCTR-311-79), International Research and Development Corporation Study No.: 450-028, January 21, 1981. M3119-79.

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General:

A pilot study was conducted to determine the dosage levels for the teratology study. The methods were similar to those previously described, except 5 animals were used to per treatment group. Pregnant Dutch Belted rabbits were orally dosed as a single daily dose on days 6 through 27 gestation. The doses were 50, 200, 400, 800, and 1200 mg/kg/day. Survival in the control and 50 mg/kg/day was 100%. All rabbits in the 800 and 1200 mg/kg/day groups died between gestation 6 and 11. In the 400 mg/kg/day group, three rabbits died between gestation days 7 and 17 and one rabbit in the 200 mg/kg/day died on gestation day 19. There were no biologically meaningful differences in appearance or behavior or mean maternal body weights in the 50 mg/kg/day. A reduction in the amount of fecal material beneath the cages of a few rabbits in 200 and 400 mg/kg/day groups wee observed. At postmodern examination, erosions of the stomach mucosa were observed in a few rabbits in 200 and 400 mg/kg/day and in a majority of rabbits in 800 and 1200 mg/kg/day. A mean maternal body weight loss occurred in the 200 mg/kg/day group during the first six days of treatment. However, weight gains were comparable to the control group over the entire treatment period. Prior to death, maternal body weight losses were noted in a majority of rabbits in 400 mg/kg/day. Mean uterine examination values for all treatment groups were comparable to the controls. There were no biologically meaningful differences in the mean numbers of corpora lutea, total implantations, postimplantation loss, early resorptions or viable fetuses in any treatment groups compared to controls. Nonviable fetuses and late resorptions were not observed in any group.

Based on these results, a dosage of 200 mg/kg/day would be considered excessive for a teratology study in rabbits with the test substance.

In addition, the main study is acceptable as a surrogate study for vinyl toluene (mixed isomers, 65%-71% meta and 32%-35% para).